New South Wales Government
Independent Planning Commission

MEG NOBLE		**OBJECT**	Submission ID: 0
Organisation:	*Garvan Institute*		
Location:	*New South Wales*	Key issues:	*N/A*
Attachment:	*Attached overleaf*		

Submission date: 12/2/2024

Dear Commissioner and Panel,

Thank you for allowing Garvan additional time to make a submission – special thanks to Kendell and Margaret for assisting me last week.

Noting the online portal is closed, please see attached Garvan and ABR's submission objecting to the proposed development for your consideration.

Kind regards,

Meg Noble

Head of Legal

Moss Vale Plastics Recycling Facility

Reference number: SSD-9409987

Submission to the Independent Planning Commission by

The Garvan Institute of Medical Research and

Australian BioResources Pty Ltd

2 December 2024

I. Executive summary

1. This submission is made by the Garvan Institute of Medical Research (**Garvan**) and Australian BioResources Pty Ltd (**ABR Pty Ltd**). ABR Pty Ltd is a wholly owned subsidiary of Garvan. The submission concerns Plasrefine Recycling Pty Ltd's (**Plasrefine**) development application (**Application**) relating to the proposed Moss Vale Plastics Recycling Facility (**Facility**).

2. Garvan owns the land and improvements at Part Lot 10, Lackey Road MOSS VALE. ABR Pty Ltd leases the facility, Australian BioResources (**ABR**), from Garvan. ABR is located immediately adjacent to the location of the Facility, the subject of the Application.

3. On 10 October 2024, the Department of Planning, Housing and Infrastructure (**Department**) referred the Application to the Independent Planning Commission (**IPC**) for determination, with the recommendation that "the project is approvable, subject to the recommended conditions of consent".[1]

4. Having reviewed the Department's "Moss Vale Plastics Recycling Facility State Significant Development Assessment Report (SSD-9409987)" dated October 2024 (**Department's Assessment Report**),[2] and the terms of the Department's recommended development consent (**Department's Recommended Consent**),[3] Garvan and ABR Pty Ltd object to the Application.

5. Garvan and ABR Pty Ltd's position is that the IPC should determine the Application by refusing consent under s 4.38 of the *Environmental Planning and Assessment Act 1979* (NSW) (**EPA Act**). In determining the Application, the IPC must take into considering matters including "the likely impacts of th[e] development" and "the public interest"

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1 "Referral letter", 10 October 2024, available at: https://www.ipcn.nsw.gov.au/cases/2024/10/moss-vale-plastics-recycling-facility.

2 "Assessment report", 10 October 2024, available at: https://www.ipcn.nsw.gov.au/cases/2024/10/moss-vale-plastics-recycling-facility.

3 "Recommended conditions of consent", 10 October 2024, available at: https://www.ipcn.nsw.gov.au/cases/2024/10/moss-vale-plastics-recycling-facility.

(EPA Act, ss 4.15(1)(b), (e), 4.40). Regard must also be had to "any submissions made in accordance with this Act or the regulations" (EPA Act, ss 4.15(1)(d), 4.40) – i.e. these submissions and the evidence in support, together with previous submissions made by Garvan (save that the position now taken is to *object* to the Application).[4]

6. Garvan and ABR Pty Ltd submit that having regard to the likely impacts of the development and the public interest, the IPC should not grant consent to the Application. That is because: (a) ABR is a one-of-a-kind facility in New South Wales, which is crucial to the achievement of the State's health and medical research priorities in supporting research infrastructure (as per the NSW Office for Health and Medical Research); (b) the construction and operation of the proposed Facility pose risks to ABR's work, which would have catastrophic adverse consequences if they were to materialise; (c) those risks, if they were to materialise, would thwart the attainment by Garvan of its statutory objects (see para 9 below); and (d) the conditions in the Department's Recommended Consent do not guard against those risks.

7. If, despite Garvan and ABR Pty Ltd's objection to the Application, the IPC were to grant consent, more stringent conditions would need to be imposed on the grant of consent than are currently included in the Department's Recommended Consent.

8. Garvan and ABR Pty Ltd has provided two statements with this submission. The first is a statement by Dr Jennifer Kingham, the Director of Animal Facilities at Garvan (**Kingham Statement**). The second is a statement by David Keenan, a consultant specialising in the planning, design, construction and operation of life science projects, including laboratories and facilities. Mr Keenan was employed by Garvan between 2004 and 2013 and had responsibilities relating to the construction of two new facilities (**Keenan Statement**).

9. The remainder of this submission is structured as follows:

   (a) **Section II** gives an overview of the work carried on at ABR;

   (b) **Section III** sets out the risks that the construction and operation of the proposed Facility pose for ABR's work; and

   (c) **Section IV** explains why the Department's Recommended Consent does not guard against those risks.

   (d) **Section V** Conclusion

II. The work of the Garvan Institute and ABR

10. Garvan is constituted under the *Garvan Institute of Medical Research Act 1984* (NSW) (**Garvan Institute Act**) (Garvan Institute Act, s 4(1)). The principal objective of the Garvan Institute is "to further knowledge in the field of human medicine by promoting

[4] On 25 November 2024, Garvan / ABR Pty Ltd foreshadowed that it would request to withdraw its submission to the IPC dated 12 November 2024. Garvan still relies on that prior submission and only withdraws it to the extent that Garvan / ABR Pty Ltd now objects to the Application.

the conduct of research in that field" (Garvan Institute Act, s 5(1)). To that end, the Garvan Institute's aims include "to discover the nature and causes of human diseases and other human inflictions" and "to improve methods of preventing, diagnosing and treating those diseases and other afflictions" (Garvan Institute Act, ss 5(2)(a)-(b)). The Garvan Institute "shall have and may exercise such functions … as are reasonably necessary for the attainment of its objects" (Garvan Institute Act, s 6(7)). Through its operation of ABR, Garvan seeks to attain those statutory objects.

11. ABR is a one-of-a-kind facility in New South Wales. ABR was built in 2008. It enables research in line with the State's health and medical research priorities which can be viewed on the webpage of the NSW Health Office for Health and Medical Research (https://www.medicalresearch.nsw.gov.au/mission-strategy/)

12. As explained on ABR's website (https://abr.org.au/):

> "Australian BioResources (ABR) is a state-of-the-art facility for breeding and holding research mice, owned and operated by the Garvan Institute of Medical Research on behalf of the medical research community in Australia.
>
> The facility provides the capacity to house the numerous genetically modified mice that underpin progress in modern medical research. These mouse colonies are critical for progress in research across all health priority areas such as the following:
>
> - Cancer
> - Mental illness
> - Arthritis
> - Asthma
> - Heart disease
> - Diabetes
> - Obesity."

13. ABR breeds and houses over 750 distinct genetically modified mouse colonies (or "lines") and a proportion of these colonies are unique to ABR (approximately 15%) (Kingham Statement, para 12). These mice colonies are critical for progress in medical research across all of the Australian Government's health priority areas (Kingham Statement, para 11).

14. There is no equivalent facility in NSW. No other facility is equipped to house breeding pairs of each mouse line. Therefore, the mice cannot be relocated (Kingham Statement, para 12).

15. Garvan is an independent, not-for-profit medical research institute. ABR mice sales are usually priced on a cost-recovery basis. If consent were granted to the Application, any increase in operational costs associated with the impacts of the Facility would be passed on to researchers who use ABR mice, including Garvan researchers. A majority of ABR customers are public universities and other not-for-profit medical research institutions and the research conducted using ABR mice would be predominantly publicly funded via

grants received from the NSW or Commonwealth governments to support the Australian Governments' health and medical research priorities.

III. The risks posed by the proposed Facility

16. As noted in the Department's Assessment Report, the mice held at ABR are "very sensitive to their surroundings" (Department's Assessment Report, para 160).

17. There are two categories and four key risks that the construction and operation of the proposed Facility pose for ABR's work. They are the risk of excess noise/vibrations (see section i. below) and the risk to air quality from a fire or other emissions (see section ii. below).

i. The risk of noise and vibrations

18. Excessive noise and vibration may impact mouse breeding and animal well-being. Mice have a hearing range of 1kHz to 100kHz range (human hearing range is 20 Hz- 20 kHz). Noise above 60 decibels in the mouse hearing range can cause an increase in cannibalism, maternal neglect and foetal loss. Similarly, vibrations in the 70-100Hz range have been shown to impact negatively on mouse behaviour (Kingham Statement, para 16).

19. As noted in the Noise and Vibration Report submitted by the Applicant in April 2024 (**Vibration Study**)[5] "the vibration velocity limit of 50 μm/s for vibration sensitive equipment is consistent with the recommended vibration velocity limit for rodent behavioural and holding rooms, as noted in the US National Institutes of Health's Design Requirements Manual." The Vibration Study includes many calculations, including for example, safe working distances for use of an 8-tonne vibratory roller with regard to various ABR buildings, but the Department's Recommended Consent does not require Plasrefine to comply with the limits set out in the Vibration Study.

20. As mentioned in Garvan's October 2023 submission, during recent construction at Braddon Road, Moss Vale staff performing micro-injections reported embryos were moving under the microscope as a vibrating compacting roller was used (Kingham Statement, para 18). This resulted in a 25 per cent loss of viable mouse embryos. Such a loss severely impacted Garvan's ability to perform that service.

21. In relation to noise, the Applicant's Environmental Impact Study Technical Report 2 Noise and Vibration[6] undertook background noise monitoring and provides a table of trigger noise levels for sensitive receivers, like ABR. The Department's Recommended Consent condition B56 does not include the same level of detail.

ii. The risk to air quality (fire and emissions)

[5] "Noise and Vibration Report" submitted by the Applicant in April 2024 available at: https://www.planningportal.nsw.gov.au/major-projects/projects/moss-vale-plastics-recycling-facility

[6] EIS "Technical Report 2 Noise and Vibration" available at: https://www.planningportal.nsw.gov.au/major-projects/projects/moss-vale-plastics-recycling-facility

22. As explained by Dr Kingham, air quality is an essential element of the ABR facility (Kingham Statement, para 20).

23. The Department has noted that "[i]f there is prolonged fire at the development during its operations, it is likely smoke and fumes would be emitted" (Department's Assessment Report, para 162). The Department has also recognised that if "fumes enter the [ABR] building via the air conditioning inlet, the mice are likely to be affected" (Department's Assessment Report, para 162).

24. The Department has noted that "FRNSW has advised that any smoke would be hot and buoyant and therefore would rise directly upwards, away from nearby sensitive receivers such as the ABR. Noting this advice, the Department finds the risk of smoke impacts on the ABR is relatively low..." (Department's Assessment Report, para 163).

25. The Applicant's Environmental Impact Study Technical Report 3 Air Quality and Odour[7] details the wind environment and states "the general pattern of wind sees the highest frequency of winds from the west, north-northeast and south-southeast (in order of prevalence). Generally, receptors downwind of the proposal in these directions are most likely to be impacted by fugitive emissions" (section 4.2.2). ABR is west of the proposed Facility (refer to figures 3.1, 4.2 and 4.3 in Technical Report 3).

26. The Executive Summary of the Applicant's Environmental Impact Study Technical Report 3 Air Quality and Odour states "the primary pollutants generated during the operation of the proposal are expected to be:
– Particulate matter from mechanical processing of plastics (e.g. crushing)
– Particulate matter, volatile organic compounds and odour from heating of plastics
– Odour from the wastewater treatment plant"

27. The ABR facility has HEPA particulate filters (Kingham Statement, para 21). Given the risks of a plastics fires and the proposed Facility's general expected pollutants and potential for noxious gas emissions, a different air filtration system (like carbon filtering), would be needed, which ABR does not have (Kingham Statement, para 22). Consequently, in the event of a fire or fugitive emissions from the proposed Facility, ABR's air intake and exhaust would have to be closed. As ABR's system for ventilating mouse cages depends on the air exhaust being active, the mouse cage ventilation system would also cease providing adequate protection (Kingham Statement, para 23).

28. Mice require active ventilation and fresh air. Noxious odours/chemicals escaping from the proposed Facility during operations or a fire could directly impact any current research being conducted at ABR involving the respiratory tract. Also, as explained by Dr Kingham, if the ventilation system is not active (due to, for example, an industrial

[7] EIS "Technical Report 3 Air Quality and Odour" available at: https://www.planningportal.nsw.gov.au/major-projects/projects/moss-vale-plastics-recycling-facility

fire), a high mortality rate of all mice would be expected within 24-48 hours (Kingham Statement, para 23).

29. Mice at ABR are currently housed in Airlaw exhaust IVC caging (Kingham Statement, para 25). At the time when ABR was constructed in 2008, Garvan /ABR Pty Ltd considered alternative caging; namely, Tecniplast Individually Ventilated Cages, which are considerably more expensive. However, given the location of ABR and the surrounding industry, the risk of potential exposure to noxious gases was determined to be low and Tecniplast Individually Ventilated Cages were not procured (Kingham Statement, para 25).

IV. The Department's Recommended Consent does not guard against those risks

30. As explained above, the proposed Facility poses risks to ABR in the form of noise/vibrations and fire/emissions. If those risks were to materialise, they could have catastrophic consequences for the mice at ABR and, in turn, scientific research in New South Wales and across Australia.

31. As explained below, the Department's Recommended Consent does not guard against the risks posed to ABR.

32. As explained below, the costs associated with Garvan mitigating the risks posed cannot be borne by Garvan or ABR Pty Ltd.

i. Risk of noise/vibrations

Proposed conditions B52, B53 and B54 in relation to construction noise and vibration

33. The Department's proposed condition B52 provides that, during the construction of the proposed Faculty, vibrations at the ABR facility must be limited to 50 μm/s:

> "B52. Vibration caused by construction at any residence or structure outside the site must be limited to:
>
> …
>
> (c) for mouse exposure (at the ABR Facility), 50 micrometres per second."

34. As noted in the Vibration Study, the Department has expressed "concerns about the effects of vibration on the adjacent Garvan Institute of Medical Research (Garvan)" (Vibration Study, p 1). The Department considered that the Environmental Impact Assessment submitted by Plasrefine relating to the proposed Facility was inadequate in that it "did not include a full assessment of construction vibration impacts on the ABR and the Department requested this be thoroughly considered" (Department's Assessment Report, para 167). Plasrefine consequently subsequently submitted the Vibration Study in April 2024.

35. The Department's Assessment Report noted that the Vibration Study "determined that vibration impacts during construction can be adequately managed" (Department's Assessment Report, para 168). The Department therefore concluded that: "To ensure

construction vibration is managed to minimise effects on the ABR, the Department is recommending a CNVMP be prepared, in consultation with the ABR, detailing implementation of all the mitigation measures recommended in the Vibration Study" (Department's Assessment Report, para 170).

36. However, none of the proposed conditions in the Department's Recommended Consent requires Plasrefine to comply with the recommendations of the Vibration Study or include those recommendations in the Construction Noise and Vibration Management Plan (**CNVMP**) to be prepared. In contrast, when Garvan led the construction of the Kinghorn Cancer Centre in Darlinghurst NSW from 2010 – 2012, finalisation of the Construction Noise and Vibration Management Plan was required before consent was granted and compliance with the plan was a condition.

37. The Department's proposed condition B53 provides as follows:

> "B53. Vibratory compactors must be limited to 8 tonnes and not be used closer than 75 metres from the ABR Facility unless vibration monitoring confirms compliance with the vibration criteria specified in condition B52 in accordance with the letter titled Response to Department of Planning and Environment issues raised - noise prepared by GHD and dated 27 February 2024."

38. Proposed condition B53 refers to the GHD report dated 27 February 2024. This report pre-dates the impacts ABR experienced to micro-injecting from the Braddon Road works. In consultation between ABR and GHD, GHD have made informal commitments to use different (smaller) machinery to that used at Braddon Road but this is not reflected in any updated reports, agreement with GHD or in condition B53.

39. The Department's proposed condition B54 provides as follows:

> "B54. The Applicant must prepare a Construction Noise and Vibration Management Plan (CNVMP) for the development to the satisfaction of the Planning Secretary. The CNVMP must form part of the CEMP in accordance with condition C2 and must:
>
> …
>
> (d) describe the measures to be implemented to manage high noise and vibration generating works such as piling, in close proximity to sensitive receivers. The measures must include special consideration for mitigation of impacts to the ABR Facility, including respite periods, timing, duration of works, and not operating the two noisiest pieces of equipment simultaneously;
>
> (e) describe a program to monitor compliance with the construction noise limits specified in condition B51 and the construction vibration criteria in condition B52. This must include details of management actions to be taken to address any exceedances, and a description of contingency measures in the event management actions are not effective in reducing noise and vibration levels to an acceptable level;
>
> (f) include strategies that have been developed with the community and the ABR Facility for managing high noise and vibration generating works, including limiting plant size, relocating equipment and stopping works;
>
> (g) describe the consultation undertaken to develop the strategies in condition B54(e);

(h) include a complaints management system that would be implemented for the duration of the development."

40. Proposed condition B54(d) states that the CNVMP must describe measures to manage vibrations, including "respite periods, timing, duration of works, and not operating the two noisiest pieces of equipment simultaneously". But it does not require the measures that are included in the CNVMP to be those set out in the Vibration Study. Similarly, proposed condition B54(e) states that the CNVMP must describe "a" program to monitor compliance. But it does not require that program to comply with the recommendations in the Vibration Study. While the recommendations in the Vibration Study are described therein as "commitments" (Vibration Study, p 13), the recommendations are not reflected in any legally binding agreement between Plasrefine and Garvan / ABR Pty Ltd. In those circumstances, it cannot be concluded that the vibration risks faced by ABR will be "adequately managed".

41. The Administrative Conditions, in particular in A10 'Evidence of Consultation' provides as follows:

> "A10. Where conditions of this consent require consultation with an identified party, the Applicant must:
> (a) consult with the relevant party prior to submitting the subject document to the Planning Secretary for approval; and
> (b) provide details of the consultation undertaken including:
> (i) the outcome of that consultation, matters resolved and unresolved; and
> (ii) details of any disagreement remaining between the party consulted and the Applicant and how the Applicant has addressed the matters not resolved.

This condition does not protect Garvan or ABR Pty Ltd in the event of any remaining areas of disagreement following consultation on the CNVMP.

42. Plasrefine must be required to comply with the recommendations in the Vibration Study (Vibration Study, pp 5-6, 13-14). Those recommendations include the following:

(a) Warning notifications at 25µm/s and stop work notifications at 50µm/s (Vibration Study, pp 6, 14 ("NV4")). As explained by Mr Keenan, these notifications should be distributed at ABR and on the site of the proposed Facility, by SMS and also visually (with strobe lights) for those working machinery (Keenan Statement, para 8).

(b) The placement of sensors in each of the following areas (using the names given to them in the Vibration Study): (i) the "Laboratory (embryo microinjection)"; (ii) "Animal holding area"; and (iii) "Stage 1 Mouse accommodation" (Vibration Study, pp 6, 14 ("NV4")). As explained by Mr Keenan, given the size of areas (ii) and (iii), there would need to be two sensors in each of those areas, one at each end of the relevant area (Keenan Statement, para 8).

43. In the Department's Assessment Report, it noted that ABR has advised it is "satisfied with the adequacy of the measures proposed to mitigate vibration impacts on the mice." (Department's Assessment Report, para 170). While ABR is satisfied with scientific

measurements used in the Vibration Study and the Technical Reports included with the EIS, the mitigation measures contained in the Department's Recommended Conditions do not incorporate detailed measuring requirement, monitoring locations and alert limits and other issues have not been addressed. In particular:

(a) The Vibration Study only recommends vibration monitoring "[d]uring road compaction works" (Vibration Study, pp 6, 14 ("NV4")). As reflected in proposed condition B52, vibrations at ABR throughout the construction of the proposed Facility must be limited to 50 μm/s. The measures recommended in the Vibration Study, including those mentioned in paragraph 31 above, must be observed *throughout* construction in relation to *all* construction activities.

(b) The Vibration Study recommends the following (not reflected in Appendix B): "Engagement and consultation with the Australian Bioresources will continue to occur to ensure vibration-intensive activities are scheduled outside of embryo microinjection activities, where possible". Garvan/ABR need this to be a requirement (rather than "where possible"). Microinjection is generally conducted 3-4 days/ week from 9am to 3pm but ABR and Plasrefine could consult and agree on a more specific schedule.

(c) The Vibration Study pre-dates the impacts ABR experienced to micro-injecting from the Braddon Road works and informal commitments since then from GHD to use different compacting equipment.

44. Given its not-for-profit status, Garvan should not have to bear the costs associated with obtaining, installing and maintaining the necessary sensors and other equipment required to monitor the Applicant's compliance with the vibration limit imposed under proposed condition B52.

Proposed conditions B52 and B57 in relation to operational noise/vibration

45. The Department's proposed condition B52 requiring vibrations at the ABR facility to be limited to 50 μm/s is only expressed to apply during the *construction* of the proposed Facility. None of the Department's proposed conditions requires that this limit be observed once the Plasrefine facility is operational. Proposed condition B57 requires Plasrefine to prepare an Operational Noise and Vibration Management Plan. However, proposed condition B57 does not require that Plan to include any requirements in relation to vibration levels.

46. It is not known what vibration levels would be caused by the proposed Facility once operational. To ensure that levels of vibrations harmful to ABR's work are not caused by the operations of the Facility, any development consent needs to include conditions relating to vibrations during the first 12-24 months of the Facility's operation. That is, the vibration limit in proposed condition B52 must be imposed for the first 12-24 months

of the Facility's operation and the measures relating to vibrations discussed in the context of proposed condition B54 above must also be complied with during that period.

47. Again, given its not-for-profit status, Garvan should not have to bear the costs associated with monitoring the Applicant's compliance in the operational phase of the Facility either.

ii. Risk to air quality (fire & emissions)

Proposed conditions B43 & B46 relating to Air Quality Discharges and Odour Management

48. The Department's proposed conditions B43 and B46 provide as follows.

> "B43. The Applicant must install and operate equipment in line with best practice to ensure that the development complies with all load limits, air quality criteria/air emission limits and air quality monitoring requirements as specified in the EPL applicable to the site. The installed equipment must be able to be retrofitted or upgraded."
>
> "B46. The Applicant must ensure the development does not cause or permit the emission of any offensive odour (as defined in the POEO Act)."

49. The proposed conditions do not sufficient impose limits and conditions with references to the data contained in the Applicant's Environmental Impact Study Technical Report 3 Air Quality and Odour in relation to fugitive emissions or other air quality hazards.

Proposed condition B62 relating to fire

50. The Department's proposed condition B62 provides as follows:

> "B62. At least one month prior to the commencement of operation the Applicant must prepare a comprehensive Emergency Plan and detailed emergency procedures to the satisfaction of the Planning Secretary. The Emergency Plan must:
>
> (a) be prepared in consultation with FRNSW;
>
> (b) be prepared in accordance with the Department's Hazardous Industry Planning Advisory Paper No. 1, 'Emergency Planning';
>
> (c) include consideration of the safety of all people outside of the development who may be at risk from the development; and
>
> (d) detail procedures for immediately notifying the ABR Facility in case of a fire."

51. In relation to the risk posed to ABR from a fire, all that proposed condition B62 requires is that a procedure be set up for notifying ABR in the event that a fire occurs at the proposed Facility. Such a notification in the event of a fire offers no protection for ABR against the risk described above (see section III.ii).

52. As explained by Dr Kingham and Mr Keenan, the risk of loss of the mice from a prolonged lack of air ventilation in the event of a fire or other air quality issue at the proposed Facility can only be averted by changes to the facilities at ABR by:

    (a) installing a suitable carbon (or other) air filtration system at ABR (Kingham Statement, para 22; Keenan Statement, para 12). The cost of purchasing such a filtration system is at least $500,000 and the incremental increase to ongoing maintenance costs are estimated to be $20,000 to $50,000 per year (Keenan Statement, para 12); and

    (b) Changing the animal caging to something similar to the Tecniplast IVCs originally considered by Garvan for the facility. The cost of purchasing such cages in 2008 was in excess of $5,000,000 (Kingham Statement, para 25). The anticipated cost now would be considerable higher than that and does not take into account other changes that may be required to accommodate a change in caging (space, racking etc).

    Again, given its not-for-profit status, Garvan should not have to bear the costs associated with upgrading the facilities at ABR to mitigate against the risks of the proposed Facility.

V. Conclusion

53. Garvan and ABR Pty Ltd make a significant contribution to biomedical research in Australia. Any impacts on the specialised mice bred at ABR will have wider impacts on biomedical research in Australia, much of which is publicly funded in the pursuit of advancements in human health to benefit all Australians.

54. This submission to the IPC objecting to the Application is made to ensure that if the Application is approved, it is not approved until strict environmental and other considerations are an embedded part of the consent granted to Plasrefine and that the consent conditions can be appropriately enforced during construction and operation.

55. Garvan constructed the ABR facility taking into consideration the environmental conditions of the location at the time. Staff and animal welfare concerns, should the Application be approved, would require significant investment to upgrade ABR.

STATEMENT OF JENNIFER KINGHAM DATED 2 DECEMBER 2024

AUTHOR DETAILS	
Name	Dr Jennifer Kingham
Occupation	Director of Animal Facilities, Garvan Institute of Medical Research
Date	2 December 2024
Contact email	[redacted]

DEVELOPMENT APPLICATION DETAILS	
Development application	Moss Vale Plastics Recycling Facility SSD-9409987
Applicant	Plasrefine Recycling Pty Ltd
Submitted by	Garvan Institute of Medical Research / Australian Bioresources Pty Ltd

STATEMENT

1 I am the Director of Animal Facilities at the Garvan Institute of Medical Research (Garvan) and oversee the Australian BioResources Pty Ltd (ABR) mouse breeding facility located at 9-11 Lackey Road, Moss Vale.

2 I am a NSW registered veterinarian, with postgraduate training in laboratory animal medicine and management. I have over 30 years' experience in the management of laboratory animal facilities.

3 Garvan's land at Moss Vale where ABR is operated directly adjoins the property that is the subject of the development application before the Independent Planning Commission.

4 I am authorised by Garvan/ABR to make this statement in relation to the proposed development.

5 I commenced employment at Garvan on 11 July 2006. ABR was designed and built in 2008 while I was an employee of Garvan and I was part of Garvan's team supporting the development in relation to the animal facilities.

6 I was also employed by Garvan when the Kinghorn Cancer Centre (TKCC) was built at 370 Victoria Street, Darlinghurst. This was a joint development by Garvan and St Vincent's Hospital (SVH), construction of which was undertaken between 2010 and 2012. Garvan led the development. Both Garvan itself and its neighbour, the Victor Chang Cardiac Research Institute (VCCRI) had concerns about the impact that noise and vibration from construction could have on sensitive scientific

equipment and animal welfare. There were several ways these concerns were managed:

a. I have read David Keenan's statement and understand there were various monitoring sensors used in the Garvan building and the Lowy Packer Building (where VCCRI are located) during construction, with limits set for both alerts and stop work.

b. In relation to sensitive scientific equipment, VCCRI had a deep sequencer (a piece of equipment used for genomic sequencing) which Garvan and SVH paid to have relocated to Sydney University for a period of 12 months. This arrangement was confirmed in a contract between the three parties.

c. In relation to the animals, one of the ways we were able to manage VCCRI's concerns was by relocating and maintaining certain mouse lines at ABR in Moss Vale. Garvan and SVH shared the costs of doing this for VCCRI which included costs of the re-delivery of mice to Darlinghurst in the event they were needed. This arrangement was also confirmed in the contract between the three parties.

7 I have been directly involved in ongoing engagement with Plasrefine, through its consultants GHD, for the past 18 months in relation to ABR's concerns regarding the development.

8 I have also been directly involved in Garvan/ABR's previous submissions in relation to the proposed development.

9 I have read the Department of Planning, Housing and Infrastructure's 'Moss Vale Plastics Recycling Facility State Significant Development Assessment Report (SSD-9409987)' dated October 2024 (DPHI Assessment Report), paragraphs 160 – 172.

10 I have also read the Department of Planning, Housing and Infrastructure's 'Recommended conditions of consent", proposed conditions B48 – B68 (relating to 'noise and vibration' and 'hazards and risk', including fire).

11 I have also read the report titled 'Response to Department of Planning and Environment issues raised – noise' prepared by GHD and dated 27 February 2024.

12 ABR breeds and houses a variety of genetically modified mice colonies (also known as 'lines') that are critical for progress in medical research across all of the Australian government's health priority areas, including cancer, mental illness, arthritis, asthma, heart disease, diabetes and obesity. Over 750 distinct genetically

modified mouse colonies are bred at any time and a proportion of these colonies are unique to ABR (approximately 15% are unique).

13 While ABR could relocate some mouse lines to Garvan, as ABR was built to be the primary mouse breeding facility, the facilities at Garvan would not be equipped to house a breeding pair of each mouse line.

14 Laboratory mice are specifically bred under sterile conditions to be free of pathogens and infectious disease.

15 Through ABR's submissions on and engagement in relation to the development to date, ABR has provided the State government and Plasrefine with information relating to the impact of exposure to noxious chemicals/fumes, and disturbance from noise and vibration, on mice breeding and behaviour.

Proposed condition B54

16 Excessive noise and vibration may impact mouse breeding and animal well-being. Mice have a hearing range of 1kHz to 100kHz range (human hearing range is 20 Hz- 20 kHz). Noise above 60 decibels in the mouse hearing range can cause an increase in cannibalism, maternal neglect and foetal loss (S Rasmussen et al 2009[i]). Similarly, vibrations in the 70-100Hz range have been shown to impact negatively on mouse behaviour (R.P. Reynolds et al 2018[ii]).

17 The Department's proposal that Plasrefine be required to prepare a detailed Construction Noise and Vibration Management Plan (CNVMP) in consultation with ABR will not protect ABR from the adverse effects of noise and vibration. When TKCC was built, a construction noise and vibration management plan was required to to be in place before consent was granted.

18 During recent roadworks at Braddon Road, Moss Vale, one of the labs at ABR that conducts embryo micro-injections was impacted by vibration caused by the use of a vibrating compacting roller. One specific ABR activity- CRISPR genome editing of mice- was found to be more susceptible to vibrations than previously thought. CRISPR genome editing, or the creation of novel genetically modified mouse strains for medical research, involves the microinjection of mouse embryos. This process is performed manually under a microscope that sits on an anti-vibration table. During Braddon Road works staff performing micro injection reported embryos were moving under the microscope. This resulted in 25% loss of viable mouse embryos.

Proposed condition B62

19 Paragraph 165 of the DPHI Assessment Report notes “[t]o ensure any potential impacts are minimised and the ABR can take appropriate and timely action to protect the mice, the Emergency Response Plan (ERP) recommended as a condition of consent would include specific procedures to notify ABR staff of any fire incident at the site.” A notification requirement may reduce the risks to ABR staff and animals from the consequences of a fire at the recycling facility, but it does not eliminate the risk.

20 Air quality is an essential element of the ABR facility and any decline in air quality has the potential to negatively impact the respiratory tracts of mice.

21 During plastics recycling there is the potential for both particulate and noxious gas emissions. The ABR facility currently has HEPA particulate filters. An increase in particulate matter in the general environment will increase maintenance frequency and costs (for example, requiring the pre-filters to be replaced more often and increased monitoring and cleaning of ABR solar panels).

22 The ABR facility does not have a carbon air filtration system, which would be required to filter noxious gases from recycling operations generally or in the event of a plastics/chemical fire.

23 In the event of heavy smoke from a nearby industrial fire, ABR’s air intake will close, as will the building air exhaust. As ventilation through the mouse cages depends on the building air exhaust, the active ventilation of mouse cages would cease. Without significant modification to the existing ABR air handling systems and filtration, high mortality in mouse cages would occur between 24-48 hours after the building ventilation is closed due to noxious smoke.

24 Data in relation to impacts to mice health depends on the insult. Noxious chemicals escaping from Plasrefine during operations could impact research involving the respiratory tract.

25 Mice at ABR are currently housed in Airlaw exhaust IVC caging, which was chosen at the time of the construction of ABR taking into account the location and surrounding industry at Moss Vale. At the time ABR was being built, Garvan/ABR considered Tecniplast Individually Ventilated Cages, but the cost is considerably higher than Airlaw. The risk of potential exposure to noxious gas was considered low, so the decision was made to use Airlaw. From my experience at that time, the cost of purchasing Tecniplast Individually Ventilated Cages for ABR in 2008 was in excess of $5,000,000.

26 During the planning and construction of the ABR facility, building air flow, ventilation and exhaust requirements were planned by the internal and external project team taking into account the location of the facility, being at Moss Vale with limited surrounding heavy industry. A building review would need to be undertaken to determine what changes ABR would need to make to its buildings to mitigate the risks posed by a plastics recycling facility being located next door.

Jennifer Kingham

Date: 2 December 2024

[i] S Rasmussen et al (2009)- Construction noise decreases reproductive efficiency in mice. JAALAS 48(4): 363-370

[ii] R P Reynolds et al (2018)- Vibration in mice: A review of comparative effects and use in translational research. Anim. Models & Exp. Med.Vol 1:2, p116-124

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Construction Noise Decreases Reproductive Efficiency in Mice

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Excessive noise is well known to impair rodent health. To better understand the effect of construction noise and to establish effective noise limits during a planned expansion of our vivarium, we analyzed the effects of construction noise on mouse gestation and neonatal growth. Our hypothesis was that high levels of construction noise would reduce the number of live births and retard neonatal growth. Female Swiss Webster mice were individually implanted with 15 B6CBAF1/J embryos and then exposed to 70- and 90-dBA concrete saw cutting noise samples at defined time points during gestation. In addition, groups of mice with litters were exposed to noise at 70, 80, or 90 dBA for 1 h daily during the first week after parturition. Litter size, birth weight, incidence of stillborn pups, and rate of neonatal weight gain were analyzed. Noise decreased reproductive efficiency by decreasing live birth rates and increasing the number of stillborn pups.

Abbreviations: dB, decibel; dBA, A-weighted noise level; L_{eq}, energy-equivalent sound level; L_n, energy-equivalent sound level where *n* represents the measurement duration in minutes; SPL, sound pressure level.

The *Guide for the Care and Use of Laboratory Animals* notes that researchers and personnel should take noise into consideration when creating and maintaining an environment for laboratory animals.[4,15,27] The effects of excessive noise can range from inadvertent triggering of audiogenic seizures to behavioral changes that could confound phenotyping or other behavioral tests.[2,3,4,6,26, 27] Studies have linked noise to stimulation of the neuroendocrine stress response system.[27] Through chronic or chronic–intermittent stimulation of the stress response system, audiogenic stressors have been linked with physiologic changes such as hypertension, cardiac hypertrophy, altered electrolyte metabolism, changes in immune responses, altered estrus cycles, decreased fertility, and increases in the number of prematurely terminated pregnancies.[17,18,20,24,26,27]

Although noise is generally considered deleterious to rodent health, the effects of noise from facility construction on rodent reproductive efficiency have not been characterized thoroughly. We sought to quantify the effects of construction noise on rodent fetal viability and neonatal growth before expansion of our institution's animal facility. Because the expansion involved building immediately adjacent to the existing vivarium and connecting the 2 buildings into a single contiguous structure (Figure 1), investigators and laboratory animal health personnel were concerned that noise from construction would decrease mouse reproductive efficiency and have deleterious effects on research. Our specific goals were to characterize ambient noise within our animal facility and noise associated with construction activities and to determine the effects of this noise on fetal viability and neonatal growth.

Sound is characterized primarily based on amplitude and frequency.[1,14,27] Amplitude refers to the 'intensity' of a sound and is measured on a decibel (dB) scale.[1,14,27] A decibel measurement is determined by taking the unit measure for sound pressure amplitude, the pascal (Pa), and converting this number to a decibel scale by using the sound pressure level (SPL). The SPL is a logarithmic scale that allows for the measurement of a large range of pressure variations detectable by the human ear.[1,14] More specifically, the intensity of sound measured in this manner is referred to as 'dB SPL.' Pitch, or perceived frequency, is a measure of how many sinusoidal oscillations occur during 1 s and is measured in hertz (Hz).[1,14,27] Factors affecting the perception of sound include loudness and pitch as well as whether the sound is transmitted through a structure or is airborne, the distance of the sound from its source, and whether any background noise (which may mask the original sound) is present. Vibration conducts groundborne noise. When interior surfaces are excited into motion by vibration, they can radiate sound.

Noise levels that are disturbing to people may not necessarily be disruptive for mice; the converse is also true. The range of frequencies readily detected by humans is between 20 Hz to 20 kHz.[13] Sound frequencies too high for human perception are defined as ultrasonic.[13] Much of the hearing range of mice (1 to 91 kHz at 60 dB SPL) is ultrasonic.[13,23] This difference in hearing ranges places much of a mouse's range above what is audible to people and renders sounds at the lower end of the human range inaudible to mice.

The A-weighted noise level, abbreviated as dBA, is a single number representing the energy sum of the noise (sound), adjusted by frequency (that is, taking into account spectral content). The frequency weighting curve (A weighting) closely represents the frequency response of the human ear to environmental noise. Even though mouse hearing is more sensitive to ultrasound than is human, most construction noise sources lack energy in the ultrasonic range (data not published[28]). Further, available instrumentation (noise loggers) and literature (equipment noise reference data) already make use of A weighting and are developed for the range of human hearing.

Before the expansion of our facility, we set forth to establish criteria for disruptive noise levels. We first characterized ambient daily noise within the vivarium and then compared this ambient noise with that of construction activities likely to cause

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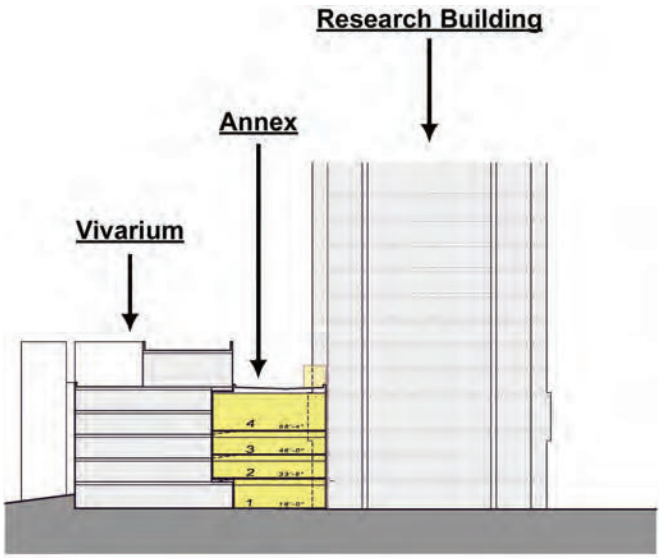

Figure 1. Expansion of the vivarium. Illustration of the future annex abridging the existing vivarium and an adjacent research building (East–West section). The annex expansion is a 4-story building designed to be contiguous with the existing vivarium.

disruption. The data from these studies allowed us to establish guidelines that set limits for construction-generated noise. In addition, we designed a composite noise barrier to help attenuate noise from outside construction activities. Because noise levels were expected to exceed our established limits even with the use of noise barriers, we designed a study to evaluate the effects of construction-generated noise on mouse reproduction and neonatal growth. Our hypothesis was that high levels of construction noise would decrease the number of live births and retard neonatal growth.

Materials and Methods

Baseline noise levels. To establish baseline noise levels within the facility, we first examined areas of the vivarium where noise likely would be most deleterious to rodent colonies. Noise-sensitive areas were determined to be Levels 2 through 5 of our building. On these levels, labs and procedure rooms were directly adjacent to the east wall of the vivarium, where the future annex would be constructed. Animal-holding rooms located toward the core of the building just west of the labs and procedure rooms adjacent to planned breakthrough sites of the exterior wall were determined to be in a region where noise levels likely would exceed established noise limits (Figures 1 and 2).

Short-term (20 min) and long-term (4 d) noise measurements (Nor140 noise monitor, Norsonic, Lierskogen, Norway) were conducted in 3 procedure rooms and 4 mouse-holding rooms, respectively. For the long-term noise measurements, noise monitors were used to log statistical hourly noise levels for 1 wk. Noise monitors were located in a central position in each room, typically on top of rodent housing racks. Short-term measurement data were acquired by digitally recording and analyzing ambient noise samples for about 20 min. All noise data were evaluated in terms of the common acoustical metric, L_{eq}, which refers to the energy-equivalent sound level. Statistical distribution descriptors, L_1, L_{10}, and L_{90}, were also used. The numerical subscript represents the measurement duration in minutes. Noise was reported by using an A-weighted sound scale (dBA). Room heating, cooling, and ventilation systems were operating in all spaces. Additional noise monitors were stationed on the exterior of the building at the construction sites to establish preconstruction outdoor noise levels over several days. In addition, the exterior shell was evaluated to determine the composite sound transmission loss provided by the concrete walls and the glass windows of the vivarium. Noise generated outside of the building was compared with that recorded inside the building to calculate noise attenuation across the concrete walls and glass windows of the vivarium.

To help establish potential effects of construction, noise monitors were placed in holding and procedure rooms during scheduled demolition of the concrete floor and slab foundation associated with an autoclave located on Level 1 of the vivarium. Short- and long-term noise monitoring measured noise transmission from Level 1 to Levels 3 through 5 during electric and pneumatic jackhammer use.

Finally noise generated by construction equipment, similar to what will be used during our building breakthroughs, was monitored and recorded. The test noise consisted of operating a hammer drill on the exterior wall and measuring noise levels at nearby locations within the building. The frequencies and intensity of the noise generated, along with the construction materials found in our facility, were used to predict sound attenuation horizontally at the site of our breakthroughs (Figure 2).

Mice. This study was conducted at an AAALAC-accredited facility after the institutional animal care and use committee approved the research project. The study population comprised 120 female Tac:SW mice (age, 10 wk; Taconic, Germantown, NY). We chose Swiss Webster mice because they segregate the *Cdh23* allele for hearing loss with no correlation between allele type and hearing function and, as a result, experience minimal age-related hearing loss.[8,9,10,22,29] Mice were provided free access to irradiated feed (LabDiet 5053, Purina Mills International, St Louis, MO) and bottles containing chlorinated (2 ppm) water. Mice were maintained in a rodent housing room in which sentinel mice exposed to dirty bedding are comprehensively screened on a quarterly basis by using serology, bacteriology, and parasitology. Mice were housed 5 mice per cage in standard-sized ventilated microisolation caging (Thoren, Hazleton, PA) on irradiated corncob bedding (Bed-o'cobs and Pure-o'cel, The Andersons, Maumee, OH) supplemented with a synthetic absorbent material (ALPHA-dri, Shepherd Specialty Papers, Milford, NJ). Bedding was changed once weekly within a ventilated cage-change station by trained animal care staff. All mice were maintained on a 12:12-h light:dark cycle; animal room temperatures ranged between 20.0 and 22.2 °C (68 and 72 °F), with relative humidity ranging between 30% and 70%. Ambient continuous noise levels in the housing room ranged between 61 and 63 dBA. Maximal measured transient noise levels in the housing rooms ranged between 80 and 87 dBA. Room air changes were set for 10.5 changes hourly, with ventilated racks (external blower motors exhausting into the building heating–ventilation–air conditioning system) supplying approximately 50 air changes hourly to each cage. After arrival at our facility, mice were allowed to acclimate for 1 wk before embryo implantation.

Embryo implantation. Personnel in Transgenic Laboratory Services (The Rockefeller University) performed embryo implantation of the Swiss Webster mice used in the gestational and neonatal growth experiments. B6CBAF1/J embryo donor mice were delivered to our facility at 4 wk of age; these mice were reported to be seronegative for *Ectromelia* virus, murine rotavirus, lymphocytic choriomeningitis virus, mouse hepatitis virus, mouse parvovirus, minute virus of mice, murine noro-

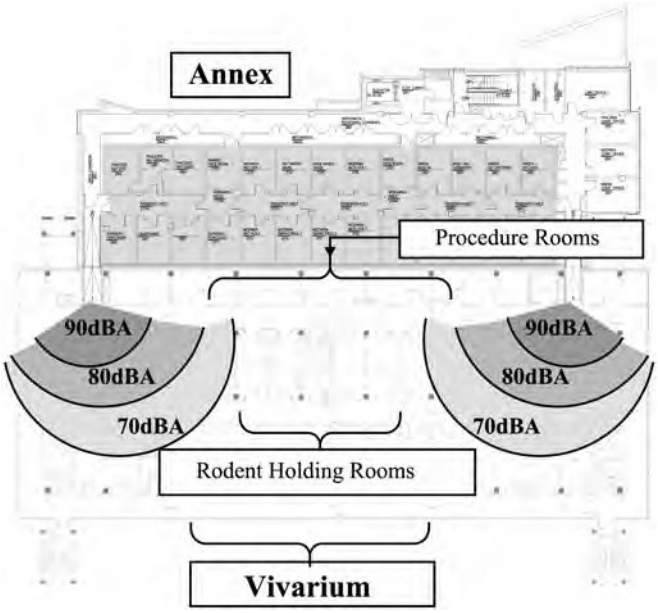

Figure 2. Noise transmission from construction into the vivarium. Conceptual illustration predicting the radius of noise attenuation to vivarium rodent-holding rooms during construction activities occurring at breakthrough points on each floor.

virus, pneumonia virus of mice, reovirus, Sendai virus, and *Mycoplasma pulmonis* and were also reported to be free of bacterial and parasitic infections. After a 1-wk acclimation period, donor mice were induced hormonally to superovulate and then mated with proven male mice (2:1 breeding ratio). Insemination was confirmed by the presence of a vaginal plug. Only 2-cell embryos were harvested and subsequently transferred to the recipient Swiss Webster mice. Prior to embryo transfer, Swiss Webster recipients were exposed to vasectomized male mice. Pseudopregnant Swiss Webster mice received tribromoethanol (5 mg/10 g body weight IP; Sigma-Aldrich, St Louis, MO), were prepared for aseptic surgery, and implanted with 15 embryos. After recovery from anesthesia, surrogate dams were returned to their home cages and were considered to be in the E2 stage of pregnancy the day after embryo transfer.

Gestational study. After embryo transfer, female Tac:SW mice were allocated randomly into a single control group of 24 mice and 4 experimental groups. The experimental groups were exposed for 1 h daily to a 6-min continuously looped audio sample of structure-borne noise from concrete saw cutting (provided by Wilson, Ihrig, and Associates, New York, NY) with dominant energy between 2 to 8 kHz. Daily noise exposure was administered between 1300 and1600 hrs. Experimental groups were as follows: group 1A (5 mice) was exposed to 90-dBA noise from the day after embryo transfer (E2) through E7; group 1B (9 mice at 90 dBA and 8 mice at 70 dBA) were exposed to noise during days E4 through E7; group 2 (13 mice at 90 dBA and 13 mice at 70 dBA) experienced noise on days E8 through E14; and group 3 (13 mice at 90 dBA and 12 mice at 70 dBA) were exposed to noise on days E15 until the end of the gestational period (E21; Figure 3).

An experimental housing cabinet with sound-attenuating properties was placed in a procedure room for use during the noise exposure study. Before experimentation, cages without mice were placed in various locations throughout the cabinet. A sound-level meter with attached microphone (Type II Sound Level Meter, model 824, Larson Davis, Provo, UT) then was placed within a cage to help to gauge sound levels and adjust speaker volume. A reference monitor (model MR5, Mackie, Woodinville, WA) was used to amplify the concrete saw sound sample and was adjusted until the noise sample could be run at both 70 and 90 dBA.

The control and experimental groups were housed in ventilated racks as described earlier. The control group was not exposed to the concrete saw noise sample and control animals were not transported to the experimental housing cabinet. For the experimental groups, the pregnant mice were left in their cages, with filter tops and water bottles removed. The cages then were placed in the cabinet for the 1-h exposure period. During the experimental phase, the sound meter was calibrated daily and was used to monitor sound output levels from the reference monitor. Depending on the subgroup, mice were exposed to either an L_{eq} noise level of 70 ± 2 dBA (range, 68 to 72 dBA) or 90 ± 2 dBA noise daily at about the same time each afternoon. After 1 h, the pregnant mice were returned to their usual housing conditions.

Mice were housed individually during the last 48 h of gestation and were observed in the morning and afternoon for signs of parturition. The number of live pups born, weight of each pup at birth, and number of stillborn pups were recorded.

Neonatal growth study. The control group from the gestational experiment was used for the neonatal growth study. Mice were monitored twice daily for signs of parturition. The number of live pups, number of stillborns, and the combined weight of all pups at birth were recorded. In addition, we tracked the individual weight gain of 3 neonates per litter from the day of birth through day 7 after parturition.

Dams with their litters remained in their home cages and were placed in the experimental cabinet for the daily 1-h exposure period. The sound-level meter (type II, model 824, Larson Davis) was calibrated daily and used to monitor sound output levels from the reference monitor (model MR5, Mackie). The control group of 9 mice and their litters were not exposed to the sample of concrete saw noise; each of the 3 experimental groups (5 dams each) was exposed for 1 h daily to an L_{eq} noise level of 70 ± 2, 80 ± 2, or 90 ± 2 dBA noise at approximately the same time each afternoon for the first 7 d after parturition. After the experiment, dams and litters were returned to their usual housing conditions. Weights of individual pups were recorded once between 1400 and 1600 each day during the monitoring period from the day of birth through day 7 after parturition.

Noise monitoring and regulation during building construction. For continuous noise monitoring throughout the construction process, noise monitors (Nor140, Norsonic) were placed at 2 locations outside the building and in at least 1 room on each floor inside the vivarium. Monitors were linked to a website (maintained by Wilson, Ihrig, and Associates) that logged ongoing noise and accommodated retrospective access of suspected noise disturbances for any given date or time during the recording process. The website also was triggered to contact specific research personnel via text message and email notifications if the levels of noise exceeded our established noise limits

Statistical analysis. Two-tailed *t* tests (Excel, Microsoft, Redmond, WA) were used to analyze numbers of stillborn pups, comparing ambient noise levels and noise treatment groups. Two-tailed *t* tests also were performed to compare the litter size of control groups with experimental groups exposed to noise during the peri-implantation period.

For neonatal growth studies, differences in weights among groups were compared in 2 ways. We first calculated the average weight of each litter for days 1 and 7, calculated the difference in

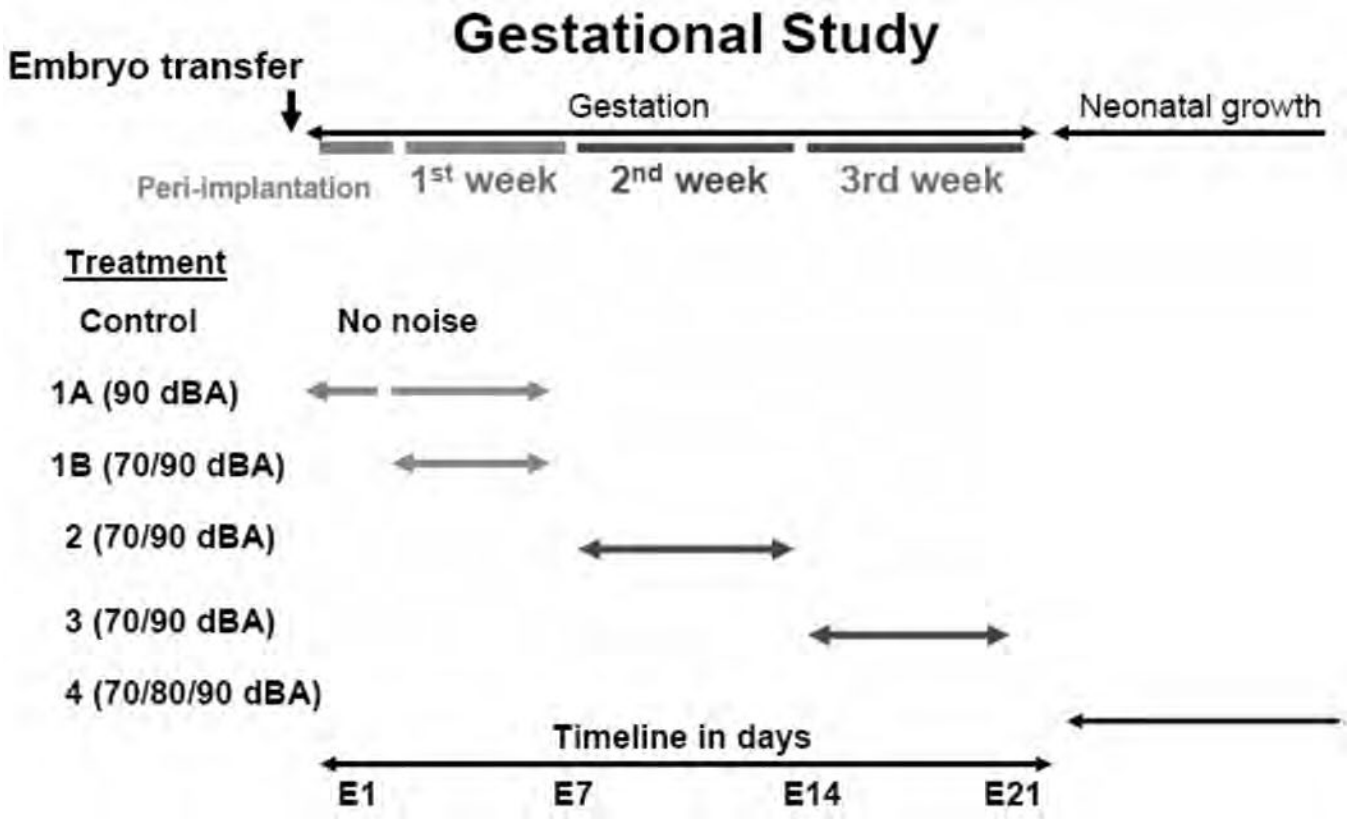

Figure 3. Experimental design. Duration and gestational timing of noise exposure. In the gestational study, noise was provided during the first, second, and third weeks of gestation (experimental groups 1B, 2, and 3). An additional treatment group received noise exposure from E2 (the peri-implantation period) through E7 (experimental group 1A). For the neonatal study, mice with litters were exposed to noise during the first week after parturition. (experimental group 4). Individual subgroups were exposed to either 70, 80, or 90 dBA.

average litter weight between days 1 and 7, and then compared these differences among groups by using ANOVA. We also calculated the individual change in weight between day 7 and day 1 for 3 pups in each litter. We examined the association of noise group on the weight change of individual pups between days 7 and 1 by using a linear regression model with general estimating equations to correct for correlations between pups within a litter (SAS version 9.1, SAS Institute, Cary, NC).

Results

Baseline noise evaluation. Ambient noise levels within rodent holding rooms averaged between 61 and 63 dBA L_{eq} with staff-generated transient noise spikes of 80 to 87 dBA L_{max} during working hours (Figure 4). The ambient noise levels on the order of 60 dBA resulted from the continuous operation of the ventilated microisolation racks. Building HVAC and rack ventilation noise was predominantly low-frequency (below 8 KHz) and therefore mostly below the hearing range of mice (Figure 5). Noise monitors were centrally located within rooms to allow for accurate comparison of ambient noise. Noise levels perceived by mice, however, may actually differ from that recorded outside their cages. Sound was attenuated by 2 dBA inside the cage when compared with noise levels immediately outside the cage.

Our analysis of a slab demolition conducted within the vivarium showed noise levels of 10 kHz and higher as the result of jackhammer use (Figure 6). Despite the high sound levels near the slab at the lower floors, substantial noise reduction to upper floors during this activity demonstrated the potential for the building to attenuate sound as it moved through the building to the upper floors of the vivarium.

Previous analysis of construction equipment used for the facility expansion demonstrated that noise levels during construction were 95 to 110 dBA and most construction equipment would have predominant energy at or below 10 kHz (data not shown). Construction equipment can generate noise at higher frequencies (above 10 kHz). However, much of the noise in the ultrasonic range (above 20 kHz) tends to be of substantially lower intensity (data not published).[28] Noise data of concrete saw cutting (data not shown) indicated relatively low levels of noise in the ultrasonic frequency range compared with the dominant saw noise energy between 2 and 8 kHz.

The exterior concrete wall provided substantial attenuation of construction noise transmitted to the interior of the building. The windows facing the construction site, however, did not have as substantial sound attenuation properties. Noise barriers made from composite materials were designed to improve the attenuation of noise transmission by the windows. The barriers consisted of 2 layers of 5/8-in. thick exterior sheathing on metal studs with mineral wool insulation. Noise barriers were installed on the outside surface of all windows facing the construction site.

Exterior to interior sound transmission studies with and without the window barriers confirmed the high level of sound attenuation achieved. In addition, attenuation was much greater for high-frequency noise than low-frequency noise, suggesting that ultrasonic noise produced outside will have virtually no effect on the rodent colonies inside the building.

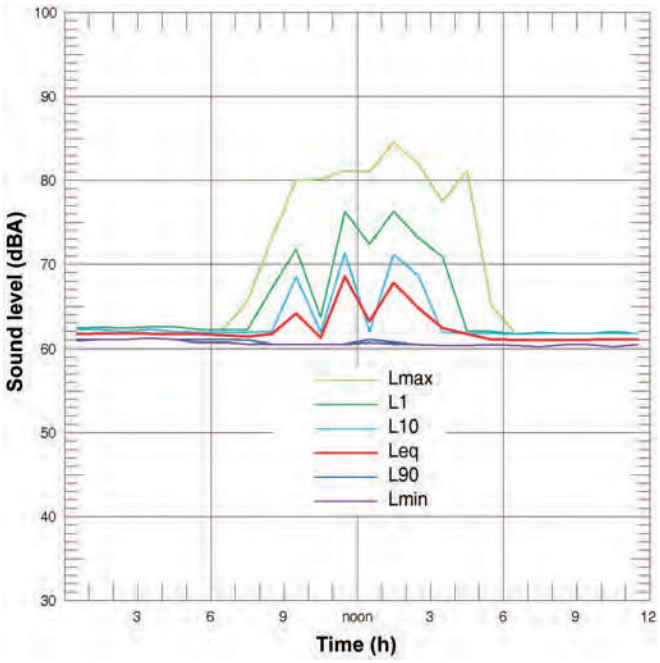

Figure 4. Ambient noise is increased due to human activity. The lines represent 24-h noise measurements taken from a rodent-holding room and are depicted as time-weighted, energy-equivalent noise levels (L_{eq}). Statistical distribution descriptors were used, L_1, L_{10}, and L_{90}, where the numerical subscript represents the measurement duration in minutes. L_{max} and L_{min} depict noise of the highest and the lowest intensities recorded during the measurement time period. Noise is increased during normal working hours, primarily because of human activity.

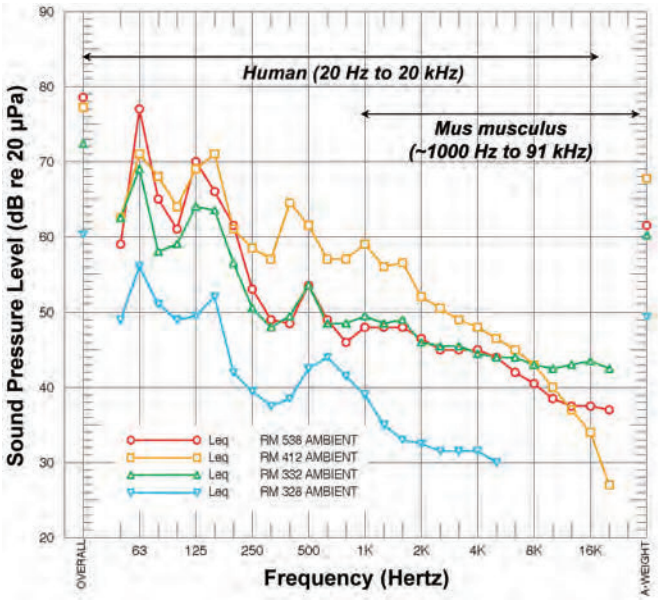

Figure 5. Ambient noise in the vivarium. These representative 24-h spectral time-weighted averages (L_{eq}) of measured noise levels were taken from 4 rodent-holding rooms. The figure illustrates the noise generated as it correlated to frequency and human and mouse hearing ranges (shown above graph lines).

Effect of noise on gestation. Only 1 of the 245 pups born to the control group of 24 mice was stillborn. In comparison, more pups were stillborn when mice were exposed to noise during the first ($P = 0.016$), second ($P = 0.024$), or third ($P = 0.031$) week of pregnancy (Figure 7). Although the effect varied among exposure groups, more pups were stillborn from dams exposed to noise of 70 or 90 dBA as compared with the control dams. In particular, the average litter size of the mice exposed to 90 dBA during the peri-implantation period (5.8 pups) was significantly ($P = 0.005$) smaller than that of controls (10.2 pups).

Effect of noise on neonatal growth. During the first 7 d after birth, the pups' weight increased over time as expected and varied depending on litter size. Growth rates of litters exposed to noise did not differ significantly when pooled weights [$P = 0.93$ (ANOVA)] or individual weights [$P = 0.64$ (linear regression model)] were compared with those of mice not exposed to noise (Figure 8).

Discussion

Our ambient noise study revealed that mice within our vivarium are exposed continuously to moderate (less than 65 dBA) levels of noise. Background noise levels found in housing rooms originated mainly from the building heating, ventilation, and cooling systems and the rack ventilation systems. This background noise is predominantly low-frequency and therefore mostly inaudible to the mice. In procedure rooms, most of the noise generated was due to human activity during normal working hours. This finding is consistent with a previous study,[27] which established that most vivarium noise either originates from personnel within the facility or is the result of animals responding to personnel within the facility.

Noise limits for construction were established based on the ambient noise levels logged in the rodent housing rooms. Because mice housed within the vivarium were maintained in an environment that routinely exposed them to moderate levels of noise, we predicted that continuous noise below 65 dBA would not have a negative effect. We established that noise should not exceed 75 dBA for 1 h and set a maximum noise allowance of 85 dBA. The 85-dBA noise limit was based on preliminary studies evaluating the behavior of nursing dams: mice exposed to 90 dBA of noise stopped nursing pups during the period of noise exposure (data not shown). Ultrasonic noise measurement data for construction equipment at close range is an area for further study because building elements such as walls, floors, or other potential transmission paths act as a mechanical filter and attenuate higher frequencies more substantially than lower frequencies.

Because noise exposure from construction activities was predicted to exceed these limits, we secured composite noise barriers over windows to increase noise attenuation across glass. The composite barriers kept noise levels within established limits during most outdoor construction activities. With the exception of a few construction activities, such as breaking through walls to connect the new building to the old, the composite barriers provided adequate sound attenuation of exterior construction noise. For the breakthroughs, supplementary noise barriers were installed inside the building to minimize noise transmission to the nearest housing rooms.

In our study, we attempted to isolate noise exposure as an independent variable in both the gestational and neonatal growth studies. Other external factors, such as cage changes, transportation of cages to the sound chamber or other environmental factors, although unlikely, may also influence fetal viability. We used a simulated noise sample as the independent variable. This simulated noise from the amplifier may have caused the mice to experience some limited mechanosensation during noise transmission, which could have contributed to the overall observed effects on fetal viability.

Our gestational study revealed a statistically significant correlation between the number of stillborn pups and noise

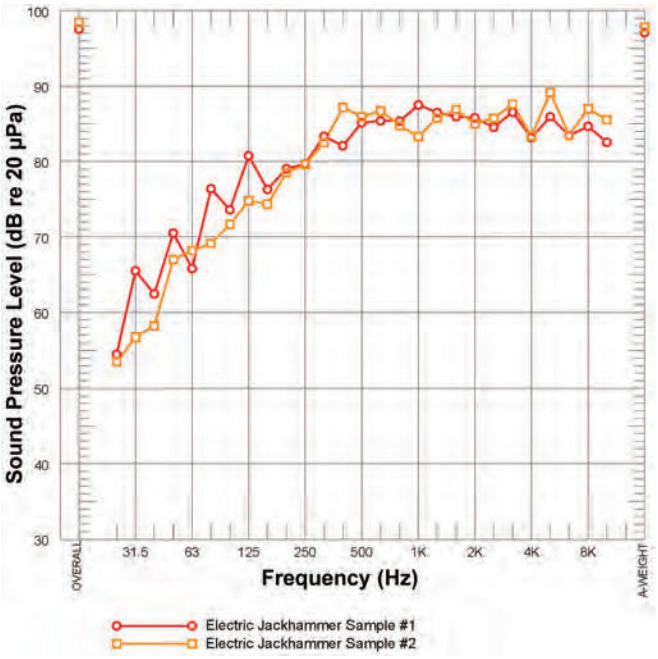

Figure 6. Noise generated from construction activities. These noise measurements were taken during a jackhammer slab demolition on the first floor. High-decibel noise was present at high frequencies (greater than 8 kHz) well within the hearing range of mice.

exposure, even at 70 dBA for 1 h daily, and noise exposure during the peri-implantation period decreased litter size. These reproductive effects could be related to a "fight-or-flight" response that noise may trigger in plasma catecholamines and the neuroendocrine system.[4,7,11,12,17,18,21,26,27] As mice are a prey species, noise is one of the first sensory systems that allows them to respond to predators.[26]

Noise exposure has been linked to increased levels of plasma catecholamines (norepinephrine and epinephrine).[11,17] In rats, norepinephrine infusion acutely reduces ovarian and uterine blood flow,[11] and in guinea pigs, infusion of norepinephrine decreases placental blood flow by 24% to 46%, depending on the dose administered.[17] Therefore, increased norepinephrine could cause decreases in blood flow that could adversely influence implantation and fetal health.

High levels of noise activate the neuroendocrine response system and increase corticosterone levels in rodents.[27] Increased corticosterone levels induced by restraint during the peri-implantation period can lead to implantation failure in rodents.[12] In addition, changes in maternal plasma cortisol levels impair fetal and placental growth in sheep.[18] Thus, fetal health could be influenced by neuroendocrine-induced changes in placental blood flow, fetal hormone levels, or placental structure.

Corticosteroids have a direct effect on estrogen and progesterone levels.[5,12] Estrogen and progesterone in turn differentially regulate the expression and secretion of inflammatory cytokines IL1α and IL6, which directly influence mouse blastocyst implantation.[5,12,16,25] In rats, restraint increases IL1 expression in the brain and IL6 expression in the liver.[12] IL1 is present in early-stage embryos and may have a role in embryo implantation.[5,12,25] IL6 reduces the rate of blastocyst attachment and embryo outgrowth in culture.[12,16] Increases in IL1α and IL6 expression as a result of noise-induced elevations in corticosterone levels may explain the reduced litter size in mice exposed to noise during the peri-implantation period.

We hypothesized that daily noise exposure would disrupt nursing or alter the maternal behavior of dams, resulting in retarded pup growth rates. However, the data revealed that 1 h of noise exposure daily at 70, 80, or 90 dBA does not significantly

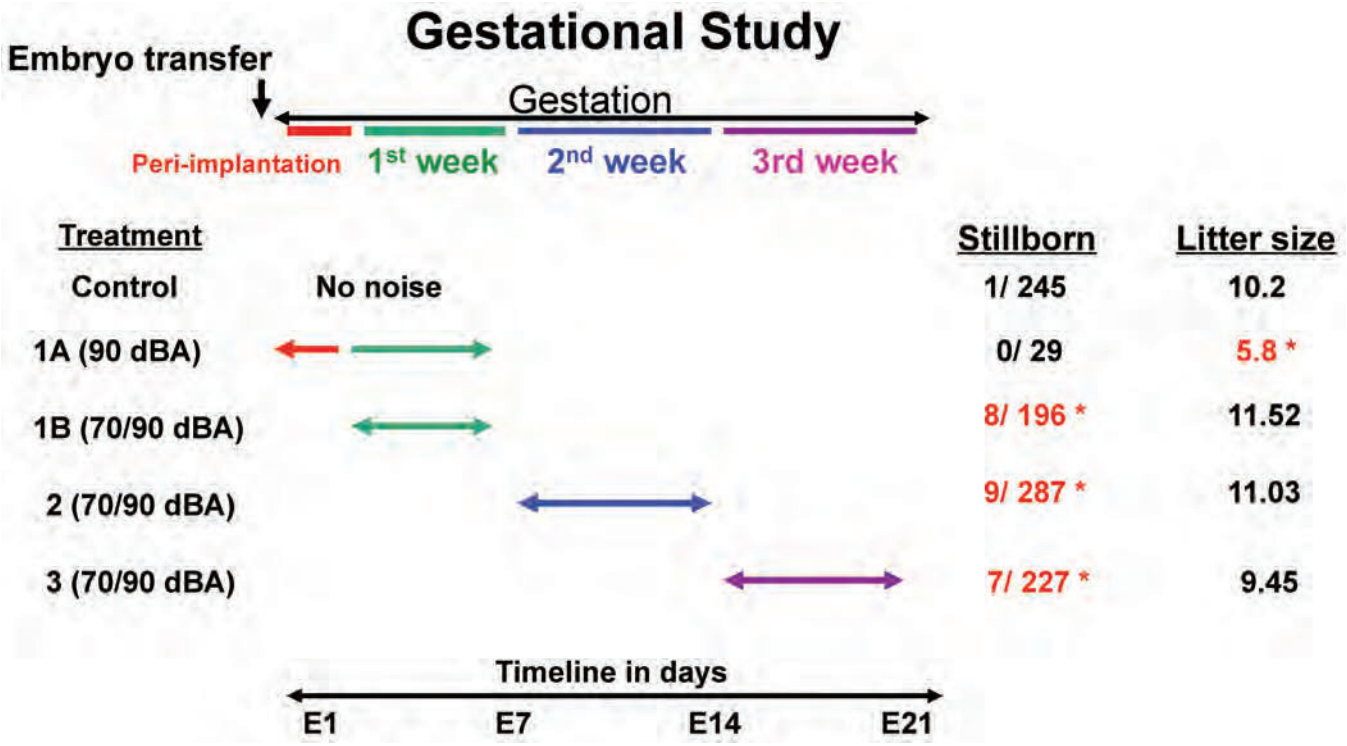

Figure 7. Noise affects litter size and the number of stillborn pups. Noise during the first (excluding the peri-implantation period), second, and third weeks of gestation increased the incidence of stillborn pups (Experimental groups 1B, 2, and 3). Noise exposure during the peri-implantation period decreased litter size (Experimental group 1A). *, $P < 0.05$.

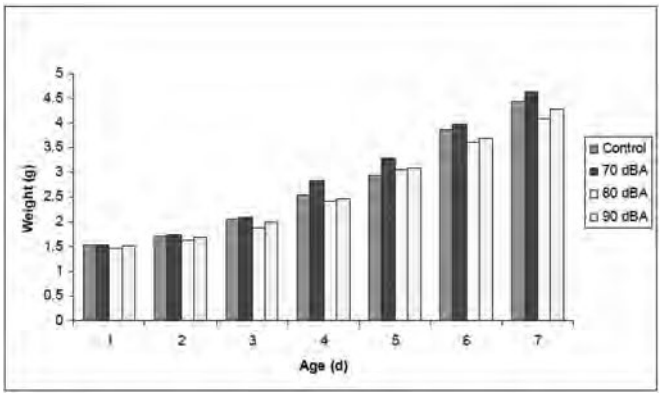

Figure 8. Growth rates of neonatal pups exposed to noise. Average growth weights of pups from similar-sized litters exposed to ambient noise (control, n = 80 pups; 70 dBA, n = 44 pups; 80 dBA, n = 60 pups; and 90 dBA, n = 52 pups). During the first 7 d after birth, the pups' weight increased over time as expected, and daily 1-h exposure to noise had no significant effect on growth rates.

alter pup growth rates. Perhaps 1 h of noise exposure does not appreciably reduce overall milk consumption over 24 h, even if the maternal behavior of dams is altered during that time, as occurred in our preliminary study. We speculate that prolonged noise exposure would decrease neonatal growth rates by altering maternal behavior enough to reduce milk ingestion by pups over 24 h, resulting in retardation of growth rates. Additional studies with longer noise exposure times need to be conducted to test this hypothesis.

In our vivarium we chose to mitigate the negative effects of noise on fetal viability by designing and placing composite noise barriers to effectively attenuate noise produced outside the building. In addition, a detailed construction schedule was developed characterizing predicted levels of noise during all phases of construction. Investigators, therefore, knew in advance when high levels of noise were going to occur so they could schedule noise-sensitive studies accordingly. Finally, noise monitors were placed at various locations on the construction site and within the existing vivarium to allow continual assessment of construction noise and to confirm that noise levels did not exceed established limits.

In conclusion, we determined that mice are exposed to moderate levels of ambient noise in the traditional housing environment of our vivarium. Taking measures to control noise during construction is important when trying to maintain mouse reproductive performance. Exposure to modest to high levels of noise, as expected during construction, significantly decreased the reproductive efficiency of mice by decreasing the number of pups born and increasing the number of stillborn pups. The observed decrease in fetal viability associated with noise exposure probably results from multiple systemic factors associated with these underlying sympathetic and neuroendocrine responses to noise. Additional studies to measure relevant stress hormones are necessary in order to better understand the physiologic mechanisms by which noise compromises fetal health.

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REVIEW ARTICLE

Vibration in mice: A review of comparative effects and use in translational research

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Abstract

Sound pressure waves surround individuals in everyday life and are perceived by animals and humans primarily through sound or vibration. When sound pressure waves traverse through a solid medium, vibration will result. Vibration has long been considered an unwanted variable in animal research and may confound scientific endeavors using animals. Understanding the characteristics of vibration is required to determine whether effects in animals are likely to be therapeutic or result in adverse biological effects. The eighth edition of the "Guide for the Care and Use of Laboratory Animals" highlights the importance of considering vibration and its effects on animals in the research setting, but knowledge of the level of vibration for eliciting these effects was unknown. The literature provides information regarding therapeutic use of vibration in humans, but the range of conditions to be of therapeutic benefit is varied and without clarity. Understanding the characteristics of vibration (eg, frequency and magnitude) necessary to cause various effects will ultimately assist in the evaluation of this environmental factor and its role on a number of potential therapeutic regimens for use in humans. This paper will review the principles of vibration, sources within a research setting, comparative physiological effects in various species, and the relative potential use of vibration in the mouse as a translational research model.

KEYWORDS
animal models, mice, translational, vibration

1 | INTRODUCTION

Translational research is commonly referred to as the combining of various scientific disciplines and using the expertise of individuals working within those disciplines to accelerate basic scientific findings into advances for novel therapeutics, medical devices, and treatment regimens for human patients.[1] Basic scientific endeavors may use various in vitro methodologies, but prior to clinical use in humans, studies in animals are imperative to fully assess diagnostic or therapeutic modalities. Animals and humans share the same organ systems, and many therapeutics and procedural regimens are comparable as well. These similarities lead to the use of animals as translational models of human disease. The animal model is selected because it is predictive of the specific disease in humans and in whole or part, the animal model will respond to medical intervention similar to humans.

Novel therapeutics require assessment of efficacy in animals, but the lack of validation of the animal model can result in erroneous interpretation of data from the model and lead to lack of predictability during extrapolation to humans.[2] Success rates of novel

therapeutics in humans during clinical development remain low due to the lack of relative levels of efficacy in preclinical testing, including animal models and in humans during clinical trials.[2,3] Careful attention to the assessment of a proposed animal model is critical to ensure species differences are identified and considered in the process. Similarly, reproducibility and transparency of published research using animals is imperative to ensure characterization of a model that will be predictive of human biology and disease.[4,5] Thus, it is critical to define the criteria being assessed within the animal model to ensure translational success in humans.

This paper reviews the current understanding of vibration in the research setting. The most recent revision of the "Guide for the Care and Use of Laboratory Animals" highlights the importance of considering vibration and its effects on animals in research.[6] Vibration likely elicits stress-mediated effects, as reported in the literature, but scant information is available on the level of vibration (threshold) that will cause effects or on the nature of the effects in animals. Understanding the threshold effects of vibration ultimately will assist in the evaluation of this environmental factor and its potential role in a number of therapeutic regimens in humans. This paper summarizes the basic principles of vibration, sources within a research setting, comparative physiological effects in various species and the potential use of vibration in the mouse, relative to other species, as a translational research model.

2 | PRINCIPLES OF VIBRATION

Sound and vibration are forms of energy that travel in waves with sound being perceived by what we hear and vibration by what we feel. In fact, sound is comprised of pressure waves caused by movement of air particles that can be detected by either a human or animal. These waves are oscillatory in nature and have both an amplitude and frequency. The amplitude contributes to the intensity of the sound or vibration and is represented by how far the peak of the wave moves past the position of equilibrium. Frequency is the amount of time that it takes to complete one cycle from a point on one wave to the same point on the next wave. The term "Hertz" is used as a unit of measure for frequency and is the number of cycles per second. One Hertz (Hz) is one cycle per second.[7] The magnitude or loudness of sound is measured in decibels, whereas the magnitude of vibration can be measured in relation to the amplitude by displacement from the point of equilibrium (often measured in millimeters), the velocity of wave movement (quantified in meters per second) or acceleration past the neutral point measured in meters per second squared (m/s^2).[8,9]

Both the magnitude and frequency of sound and vibration are important in the perception and potential adverse or therapeutic effects in humans and animals. For example, the human hearing range is from 20 Hz to 20 kHz and the mouse hearing range is from about 1 kHz to 100 kHz.[10] Likewise, an object will vibrate differentially based on its physical composition and will also tend to vibrate at some frequencies more than others. The frequency where vibration occurs most readily and can amplify the vibration is called the resonance frequency. The resonance frequency is located within the resonance frequency range (RFR), where the vibration would become greater at frequencies closer to the resonance frequency and somewhat less at the ends of the range. These frequency ranges are unique to an animal, a body region, or any other object and are dependent on that subject's physical composition with regard to "stiffness" and mass. Any object or part of an animal's body has a resonance frequency (Fn), which is calculated by the formula $Fn = 1/(2\pi) \times \sqrt{(k/m)}$, where k is the stiffness constant, and m is the mass.[11,12] Knowledge of resonance frequencies is important because vibration near these frequencies, compared with other frequencies, will be perceived more strongly and ultimately will induce more physiological effects, including those considered harmful.[13] Therefore, different species or size of animals may perceive vibration to a lesser or greater degree depending on the frequency of the vibration. In addition to the frequency of vibration, other factors that will determine the effects on animals include magnitude, duration, whether the vibration is directed at the whole body or is localized, and potentially individual variation in perception across species or within the same species.

Because both frequency and magnitude impact the exposure level of vibration, the interpretation of the literature with regard to both beneficial and adverse effects of vibration can be difficult. Until recently, only the resonance frequency of the liver had been determined in mice, which is 2-7 Hz.[14] The predicted resonance frequency for the mouse was calculated[11(p655)] and then studied by performing measurements in both rats and mice.[15] Importantly, the resonance frequency for a single organ is quite different than the vibrating frequency of the entire body in cumulative. Similarly, different body regions of a human or animal will have different RFRs. For example, the human abdomen has a resonance frequency of 4-8 Hz, the thorax of 5-10 Hz, and the head from 20 to 30 Hz.[16] In rats, the RFR was 27-29 Hz for the abdomen, 225-230 Hz for the thorax, and 75-80 Hz for the head.[17] Although resonance frequencies had not been reported for mouse anatomical regions, the predicted RFRs for mice were 85-92 Hz for the abdomen, 711-727 Hz for the thorax, and 237 to 253 Hz for the head when assuming equivalent inherent stiffness of tissue is similar in mice and humans.[11(p655)] Anesthetized mice that were exposed to vibration generally attenuated vibration that would have been detected by a mounted accelerometer on their back, except for vibration in the ranges of 30-100 Hz. Instead, the magnitude of the vibration in these ranges was either equal to or greater than the applied vibration, indicating that the RFR for these animals lie within these ranges.[15(p1963)] Mice that were exposed to vibration at 80 and 90 Hz showed increases in blood pressure and/or heart rate, whereas no increases were observed with frequencies 70 Hz or less or with 100 Hz or greater.[18] A recent study has demonstrated that mice show more behavioral alterations due to whole- body vibration (WBV) predominantly between the frequencies of 70-100 Hz.[19] Therefore, mice appear to be the most sensitive to vibration between frequencies of 70-100 Hz. Within this RFR, mice should be most susceptible to low

level vibration, which would likely most affect an animal's normal physiological and behavioral functions.

3 | SOURCES OF VIBRATION IN RESEARCH SETTING

Because the care of animals requires the use of mechanical systems and equipment, vibration will be present in the animal facility to some degree. There are three general sources of vibration: vibration produced from mechanical systems or procedures within the animal facility; vibration produced outside, but near the animal facility; and vibration resulting from the transportation of animals from the vendor or to locations within an animal program. Sources of vibration that occur within the animal facility include ventilation systems, husbandry-associated cleaning and sterilizing equipment, ventilated racks, and cage change stations.[11(p656),20] There are several studies regarding the numerous effects of construction noise and vibration on rodents that have detailed effects such as increases in corticosterone levels and other alterations in biochemical parameters or reproductive efficiency.[21-28] Recently, studies have begun to separate the effects of construction noise vs vibration. In one study, dams of two strains of mice were exposed to vibration levels comparable to that produced in an animal facility from proximal construction.[23(p3)] While no changes in overall fertility were noted, nursing dams did show some alterations in normal maternal behavior. The study raised several important points to consider with regard to construction-induced vibration. Specifically, vibration from outside sources (ie, construction, trains) is often produced in sudden, intermittent bursts in contrast to vibration produced over long continuous time periods. Intermittent vibration is thought to produce more adverse effects than continuous vibration due to its unpredictability.[23(p3)] While no changes in fertility were detected in this study, other research has demonstrated increased rates of abortion, cannibalism, and resorptions following construction procedures in proximity to animal facilities.[29] High rates of cannibalism were also observed in a mouse housing room located near an active railroad.[22(p737)] Measurements were taken to adequately characterize the frequency and magnitude of both the sound and vibration produced by the passing train. While most sound that was produced was outside the range of mouse hearing, significant vibration of up to 0.25 m/s^2 was generated. In addition, the exposed female mice exhibited higher corticosterone levels relative to female mice that were not vibrated.[22(p737)] Lastly, the transportation of animals by vehicle, by a hand-pushed cart, or by hand has been shown to produce a relatively high degree of vibration exposure.[30,31] Using an accelerometer placed inside a standard polycarbonate mouse cage, vibration was measured during transportation by either hand-carrying or with several types of carts. With transport of the cage along a set pathway, vibration within the cage varied by as much as 35 m/s^2 between the transportation methods, suggesting that movement of animals even between rooms and buildings, which is common in many research environments, can subject animals to considerable vibration.[31(p544)] For this reason, animals should be provided with an opportunity to recover from vibration exposure before being used in scientific experiments. Mice that were transferred from their housing room to another room across the hall and placed on a shaker apparatus, with no vibration administered, took between 1.5 hours to approximately 24 hours for their active behaviors (eg, locomotion, rearing, sniffing) and inactive/maintenance behaviors (eg, sleeping, grooming, eating) to return to pre-transport levels.[19]

While it is not always possible to completely mitigate vibration from sources such as trains, subways or proximal construction, these factors should be taken into consideration during the design and location site planning for animal facilities. In addition, care should be taken to reduce vibration from cage movement and disturbances within the animal room or between locations within an institution. Even when rodents are exposed to movement from opening cages for routine experiments or normal husbandry activities, animals may be stressed. For example, rats have been shown to have higher corticoid metabolites in their feces following husbandry procedures.[32] Appropriate training of research personnel and staff can help mitigate some of these effects with proper handling. Even simple measures and policies, such as limiting cell phone use in animal facilities can have an effect. In a study with rats, exposure to intermittent noise and vibration from cell phones increased anxiety-like behavior during plus maze testing.[33] Vibration-induced effects should also be considered when obtaining materials and equipment for animal facilities. For instance, most modern individually ventilated racks have a heavy construction with clips to hold cages in place. Such racks may be better at dampening short bursts of vibration compared to other types of racks. In addition, in one study that looked at vibration produced by common transport carts used in a facility, metal carts with large wheels helped to decrease vibration at the cage level. Using padding on the carts also helped to further dampen vibration's accelerative forces.[31(p546)]

4 | ADVERSE VIBRATION EFFECTS AND POTENTIAL BENEFITS IN ANIMALS AND HUMANS

In humans excessive vibration can cause effects on bone, joints, nerves, muscles, and blood vessels that can be profound and debilitating.[34,35] Because of these effects, regulations and standards have been employed to limit vibration exposure in humans.[36,37] Similarly, animal studies have shown that vibration can have a myriad of adverse effects in many different species, including altering the normal physiology and even cell structure. Information regarding the adverse effects of vibration in animals and humans is summarized in Table 1.

Stress as a result of vibration, not unexpectedly, causes increases in heart rate in mice and humans. Conscious mice exposed to vibration can exhibit increases in heart rate (HR) and mean arterial blood pressure (MAP). When mice were anesthetized and unconscious,

TABLE 1 Adverse effects of vibration in various species

Species	Adverse effect	References
Mouse	Decreased the number of litters born relative the number bred	22(p737)
Mouse	Nursing dams exhibited noticeable agitation and disruption in nursing	23(p8-10)
Mouse	Increased both heart rate and mean arterial blood pressure	18(p374,375)
Mouse	Decreased the number of blood vessels per muscle fiber in the soleus muscle	66
Mouse	Startle response and fear-related behaviors	19
Mouse	Increased blood levels of corticosterone	22(p737)
Mouse, Pig	Changes in reproduction associated with hormonal changes with an increase in stress hormones	Mouse 22(p737), Pig67
Rat	Disrupted myelin in axons, decreased the arterial lumen size, and an increased arterial smooth muscle vacuolization in the tail	68,69
Rat	Altered serotonin levels in the brain	70(p15)
Rat, Dog	Caused stress leukograms	Rat71, Dog72
Dog	Increased aortic flow rate and pulse pressure during anesthesia	40
Rabbit	Alterated neuropeptides in the dorsal root ganglion associated with ultrastructural changes in cellular structure	73

neither HR nor MAP were elevated under the same vibratory conditions, suggesting that consciousness is a requisite for these cardiovascular effects in mice.[18(p374,375)] To assess the effect of noise and vibration on heart rate in humans, study participants were exposed to experimentally induced vibration, equivalent to that produced from a train, during sleep. In 79% of participants subjected to the high-vibration condition, an average increase of at least 3 beats per minute per train was observed and cardiac responses were generally higher in the high-vibration condition than in the low vibration condition.[38] The increased HR in humans was characterized by an initial and then a delayed response, indicating that a startle response was associated with awakening and a more conscious response ensued as the vibration continued. Similarly, the HR of participants receiving vibration during squat training had higher HR than individuals not receiving vibration. The HR of individuals that received vibration was increased on the initial training day and declined during subsequent training days, showing a rapid cardiovascular adaptation to the vibration stimulus.[39] Therefore, both humans and mice may perceive vibration as a psychological stressor and subsequently undergo increases in HR. However, vibration may have other cardiovascular effects that do not require consciousness since vibration at very high magnitudes (9.8-29.4 m/s^2) caused an increase in aortic blood flow and pressure during anesthesia in dogs and pigs.[40(p386)]

In larger species, vibration associated with transportation is considered one of the factors involved in transportation stress.[41] Exposure of swine to WBV, to mimic transportation stress, caused behavioral avoidance of the vibration produced.[42] Transportation-induced vibration in poultry causes stressed-induced behaviors and the stress-related effects of increased heart rate and blood circulation.[43] Vibration levels during transport can become high, which may contribute to observed behavioral alterations. The vibration levels produced from routine animal facility transport methods such as carts and hand carrying have been measured.[31(p544)] In some instances, vibration magnitudes reached as high as 17.31 m/s^2 for some of the carts tested.[31(p546)] These levels are much higher than ambient vibration levels of approximately 0.024 m/s^2 measured in animal rooms.[11(p655)]

Some studies have shown potential benefits of vibration on bone, muscle, fat accumulation, metabolism, and in wound healing (Table 2). The studies demonstrating the positive effects of vibration point to exciting potential for vibration to be used in the therapy for conditions that affect humans as well as areas for future translational studies using animal models. Because of the potential positive effects, vibration has been used to treat musculoskeletal diseases as well as to increase athletic performance in humans. Work still needs to be done, however, to determine the accelerations and frequencies that are most beneficial.[44,45] As discussed below, because the frequency, magnitude, and duration of exposure can determine if vibration will have negative, positive or no effects, animal models will be important in developing these therapeutic uses.

5 | CHALLENGES IN ANIMAL STUDY DESIGN

Because of the varied nature of experimental design applied to WBV studies reported in the literature, it is challenging to determine which vibration protocol is likely to have the greatest benefit, adverse effects, or no effects at all. For example, in studies to use vibration exposure for promoting bone growth or maintenance, there were acceleration ranges between 2.94 and 29.43 m/s^2, frequency ranges between 8 and 90 Hz, varied durations of exposure, as well as animal age and species.[46(p1059),47(p349),44-46,48,49] Higher magnitude WBV of 19.62 and 29.43 m/s^2 was only osteogenic in ovariectomized rats,[50(p316)] whereas low magnitude vibration applied to osteoporotic (ovariectomized) rats at approximately 2 m/s^2 reversed some of the negative effects of osteoporosis and accelerated early peri-implant osseointegration.[51] An evaluation of WBV effects on

TABLE 2 Potentially beneficial effects of vibration in various species

Species	Potentially beneficial effects	References
	Bone	
Mouse	Increased bone formation on the endocortical surface of the metapaphysis during skeletal growth	74
Mouse	Increased cortical bone area and cortical thickness in the femur and tibia diaphysis	75
Mouse	Increased trabecular metaphyseal bone formation and percentage of mineralizing surfaces	76
Mouse	Increased trabecular bone volume of the proximal tibial metaphysis	77
Rat	Mitigated negative effects of bone repair and bone callus formation due to ovariectomy	78
Rat	Improved fracture callus density, enlarged callus area and width, accelerated osteotomy bridging, upregulated osteocalcin expression and suppressed osteoclast activity after ovarectomy	79
Rat	Improved stiffness and increased endosteal and trabecular bone densities during fracture repair after pharmacological induction of osteoporosis and ovariectomy	80
Rat	Attenuated the loss of bone mass and trabecular bone microstructure after spinal cord injury	81
Rat	Promoted migration of mesenchymal stem cells and fracture healing, upregulation of several osteogenic proteins, up-regulation of the expression of chondrogenesis-, osteogenesis-, and remodeling-related genes	82-84
Sheep	Increased femoral trabecular bone formation	47,85
	Muscle	
Humans	Prevented a shift in myofiber type during extended bed rest	86
Humans	Increased isometric muscle strength, explosive muscle strength, and muscle mass in men older than 60 y of age	87
Human	Caused muscle relaxation in the neck and back	88
	Other effects	
Mouse (diabetic)	Attenuated hyperglycemia and insulin resistance, reduced body weight, normalized muscle fiber diameter, mitigated adipocyte hypertrophy in visceral adipose tissue, and reduced hepatic lipid content	89
Mouse (diabetic)	Decreased skin wound healing time, increased wound –associated angiogenesis and granulation tissue formation, accelerated wound closure and re-epithelialization, and increased expression of insulin-like growth factor-1, vascular endothelial growth factor and monocyte chemotactic protein-1 in the wounds	48
Humans	Increased the oxygen carrying capacity of the blood during exercise	49

bone formation in healthy rats using a constant acceleration and 45 or 90 Hz demonstrated that only a frequency of 90 Hz stimulated bone formation,[46] indicating that studies performed only at the low frequencies would have yielded a different conclusion regarding the effects of vibration. Although there have been varied experimental regimens used in vibration research, some consistency in findings is starting to emerge. For example, a second study has demonstrated that WBV at 90 Hz stimulates trabecular bone cellular activity, accelerates cortical bone growth, and increases bone mineral density in mice.[52] The WBV of 90 Hz is consistent with our established RFR for mice.[15(p1963)] Previous studies have been conducted without regard to the RFR of the animal and thus, the results may have been different if a frequency within the RFR had been used. Therefore, when designing vibration studies in animals careful consideration should be given to the frequency used as well as the magnitude.

There are also species considerations in animal study design. For example, techniques to study the effects of vibration at the molecular level are more available in mice than non-rodent species. Rats, however, may be a more appropriate rodent model for some studies, such as the study of vibration effects on the tail blood vessels and nerves, since they are larger in size. Rats share the same advantage as mice in that larger numbers can generally be used due to lower cost, reduced space requirements, rapid generation time, and increased availability.

6 | USE OF VIBRATION IN ANIMAL MODELS

The effects of vibration in animals is varied and can be either destructive or beneficial, likely depending on magnitude, duration, whole-body or localized, and presumably the sensitivity to the vibration for the species. The use of the mouse as a model to study human conditions has the advantage that transgenic, knock-out and knock-in strains are available to delineate the function of various genes in contributing to the harmful or beneficial effects of vibration in humans.

Vibration- induced effects in people include hand-arm vibration syndrome (Raynaud's phenomenon) consisting of vasospasm in hands and fingers,[53] lower back pain,[54] motion sickness, bone damage, varicose veins/heart conditions, stomach and digestive conditions, respiratory effects, endocrine and metabolic changes, impairment of vision/balance, and reproductive organ damage.[55] In mice, vibration-induced effects have been demonstrated in bone, muscle, hormones, metabolism, and reproduction as well as altering cardiovascular parameters, causing weight loss and increasing stress.[44,56] The mouse, therefore, is a valuable model to study many of the adverse conditions caused by vibration in humans.

In both humans and animals, diminishment of skeletal strength and muscle atrophy can lead to decreased mobility and function.

However, the musculoskeletal system responds to dynamic load in an anabolic manner and vibration therapy may serve to augment pharmacological therapy to strengthen bone and muscle.[56] The musculoskeletal system is able to tolerate a high level of vibration without damage due to its inherent elasticity and plasticity of the system, including the natural shock absorbers of the articulating joints. As previously noted, vibration has shown positive effects on both muscle and bone in mice, and therefore, the mouse model would be useful in the study of muscle and bone health.

Osteoporosis or bone fracture repair is another area where vibration may be beneficial and rodents may serve as a translational model. However, in humans, both osteoblastic and chondroblastic osseous repair occurs, while endochondral bone formation predominates in rodents. Fracture repair of the long bones in animal models has been well described, but vibration was not assessed as an adjunct to traditional intervention.[44] Considerable variation in bone morphology and healing processes exist among animal species; thus, characterization of each model is critical to appropriately correlate experimental outcomes to a skeletal condition in human. The bones in larger species (eg, canine, caprine, ovine swine, and nonhuman primates) do not undergo the continuous growth or modeling observed in rodents, while fracture fixation methods and biomechanics of fractures in these larger species mimic those used in humans.[56] Thus, preclinical research is commonly performed in these larger species instead of rodents. Despite this difference in bone healing, 53% of animals used in fracture studies over a 10-year period were either rats or mice and the large percentage of rodents used correlates to their applicability to molecular biology techniques, the ability to use a larger number of animals, and faster healing rates.[56]

Experimentally induced vibration has been used commonly in various behavioral, physiological, and psychological research models for decades as a source of stress.[57-59] In these studies, stress is defined as a physical, chemical, or emotional factor that causes physical or mental tension.[58] Often stress is a chronic condition and animal studies utilizing vibration are an important part of modeling the pathological effects of stress. Depending on the model, use of vibration or shaker stress often may prove advantageous over other models of induced stress such as physical restraint, foot shocks, or forced-swim testing in rodents. Use of shaker stress in animal studies provides a mild form of stress that has been used reliably to induce a form of stress that results in changes in blood pressure, heart rate, and stress hormones. Since shaker stress can be delivered remotely to an animal's home cage, it reduces the potential for artificial enhancement of the stress response from factors such as handling, restraint, noise, or pain.

Some of the most common models that utilize shaker stress are those used to study conditions such as depression and post-traumatic stress disorder (PTSD).[58,59(p320)] PTSD affects nearly 10% of Americans, but finding appropriate animal models is difficult due to the co-morbidities PTSD shares with other conditions such as anxiety and depressive disorders.[59] It is important for animal models to exhibit similar underlying characteristics or components of the corresponding disorder being studied. This allows for adequate study of the various factors that may contribute to disease processes, such as genetic or environmental factors. It also ensures that more reliable predictions are made about treatment effects. A study of rats exposed to intermittent shaker stress as part of a chronic stress schedule assessed the effects of the chronic unpredictable stress on anxiety-like behavior and cognitive deficits.[59(p320)] In conditions such as depression, human patients can also display cognitive changes.[60,61] Rats exposed to chronic unpredictable stress displayed cognitive deficits and increased anxiety similar to effects seen in the human condition. Rats also showed improvement in cognitive deficits when common treatments were tested, such as selective serotonin reuptake inhibitors and other drugs, indicating the appropriateness of the model.[59(p320)] Because shaker stress has also been shown to cause stress in mice and induce behavioral changes,[19] vibration in mice may also provide an appropriate stressor for the study of anxiety and depression.

The availability and current use of many genetically altered strains of mice offer a wide array of potential mouse models of human disease. For example, shaker stress has been used to study how early development factors affect the stress response in later life. In one study, progeny from NOS-3 knock-out mice were exposed to shaker stress to determine how the intrauterine environment affects the cardiovascular response to stress. NOS-3 is an enzyme responsible for the generation of nitric oxide in endothelial cells. Nitric oxide is a smooth muscle relaxant that plays a vital role in maintaining uteroplacental perfusion via vasodilation. NOS-3 deficient knock-out mice are susceptible to hypertension and reduced fetal growth during gestation. In the study, mature mice born to NOS-3 knock-out dams had greater changes in blood pressure in response to intermittent two-minute shaker sessions that were repeated over 24 hours relative to wild type mice.[62] Other studies have used shaker stress to study the interplay between circadian patterns and cardiovascular responses to stress.[63(p768)] All of these animal models are valuable tools in advancing the knowledge of the numerous factors that determine how stress affects various disease processes in humans.

Mice may also serve as a good model to study the potential of vibration as a therapy for wound treatment. Because local vibration has been shown increase blood flow in the skin of humans, it has been proposed as a treatment for pressure ulcers or other skin wounds.[64] Pressure wounds and other skin injuries may be more prevalent or of concern in diabetics. Because wound healing time in diabetic mice decreases when vibration exposure occurs,[48] the mouse model needs to be explored further with regard to wound healing.

There is evidence that vibration therapy may be beneficial in many age-related conditions.[65(p319)] WBV has been suggested to attenuate muscle atrophy resulting from bed rest, and may increase postural balance and gait. Similarly, exercise supplemented with WBV increases muscle strength and speed in older women following 24 weeks of treatment. Mice could play a very valuable role in studying the effects of vibration to prevent or treat conditions related to age.

7 | SUMMARY

Vibration experienced by animals can elicit stress-mediated effects and increased emphasis is being placed on vibration with regard to the welfare of animals and as a research variable. To understand the threshold for these effects, the sensitivity of a species to vibration is crucial to determine the utility of the animal as a translational model that is predictive in humans for a therapeutic effect. The mouse is a commonly used model in biomedical research, particularly when investigating molecular and cellular effects. This species, through genetic engineering and humanization, is appropriate for investigating the effects of vibration in a number of therapeutic modalities. There are numerous effects of vibration on the mouse, both those considered adverse as well as those with the potential to be used as a translational model for human therapeutics. Continued characterization of the effects of vibration in the mouse model will facilitate its use as a translational model for various therapeutic endeavors.

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STATEMENT OF DAVID KEENAN DATED 2 DECEMBER 2024

AUTHOR DETAILS	
Name	David Keenan
Occupation	Consultant / Project Advisor
Date	2 December 2024
Contact email	david.keenan@danbarconsulting.com.au
DEVELOPMENT APPLICATION DETAILS	
Development application	Moss Vale Plastics Recycling Facility SSD-9409987
Applicant	Plasrefine Recycling Pty Ltd
Submitted by	Garvan Institute of Medical Research / Australian Bioresources Pty Ltd

STATEMENT

1 I am an experienced Project Advisor, with over 20 years' experience. I specialise in advice relating to the planning, design, construction and operation of life science projects, including laboratories and facilities. I presently work at Danbar Consulting Services. I am a scientist by training. I hold an honours degree in applied zoology and, earlier in my career, I worked as a research scientist.

2 I started worked at Garvan on 29 June 2004 until 1 May 2013. I was employed full time by the Garvan Institute of Medical Research (**Garvan**) as the Operations Manager in the Building Operations Division. Since I left Garvan in 2013, I have done consulting work for Garvan on two occasions: (i) in 2018; and (ii) I commenced a piece of consulting work for Garvan approximately six weeks ago, which is unrelated to the development proposed by Plasrefine.

3 During my time as an employee of Garvan, I had responsibilities relating to the construction of two facilities, each of which is located near Garvan's principal place of business at 384 Victoria Street, Darlinghurst:

a. The first was the Lowy Packer Building at 405 Liverpool Street, Darlinghurst, which houses the Victor Chang Cardiac Research Institute (**VCCRI**). The Lowy Packer Building was a development by the VCCRI and several St Vincent's Hospital research groups, which was constructed between 2006 and 2008. Garvan had animal management concerns in relation to the development. Garvan's principal place of business houses mice, some rats and at that time, zebrafish, and is located next door to the Lowy Packer

Building. I had the responsibility for representing Garvan's interests in relation to this construction project.

b. The second was the Kinghorn Cancer Centre at 370 Victoria Street, Darlinghurst. This was a joint development by Garvan and St Vincent's Hospital, construction of which was undertaken between 2010 and 2012. Garvan had the lead role in relation to the development, and I was Garvan's Project Director for the project. Both Garvan itself and VCCRI had animal management concerns in relation to this development. VCCRI's Lowy Packer Building houses mice, rats and zebrafish.

4 From my time as an employee of Garvan, I am also familiar with the Australian BioResources facility (**ABR**). ABR was designed and built while I was an employee of Garvan. I was part of Garvan's project team supporting the development. I understand that the buildings making up the ABR have not changed since I was an employee of Garvan.

5 I have been provided with and have read the following materials:

a. paragraphs 160 – 172 of the Department of Planning, Housing and Infrastructure's 'Moss Vale Plastics Recycling Facility State Significant Development Assessment Report (SSD-9409987)' dated October 2024;

b. the proposed conditions of development consent relating to 'noise and vibration' and 'hazards and risk' (including fire) (proposed conditions B48 – B68) in the Department of Planning, Housing and Infrastructure's 'Recommended conditions of consent';

c. the letter titled 'Response to Department of Planning and Environment issues raised – noise' prepared by GHD and dated 27 February 2024 (referred to in proposed condition B53) (**GHD Letter**). While referred to as a 'letter' in the proposed conditions of development consent, this is a 14-page report;

d. the Statement of Dr Jennifer Kingham dated 2 December 2024.

6 From my practical experience delivering construction projects adjacent to small animal research facilities similar to ABR, through both the delivery of the Lowy Packer Building and the Kinghorn Cancer Centre, I have an understanding of the risks for small animals associated with construction activities and what is necessary to manage and mitigate those risks. In particular, as a result of the construction of the Kinghorn Cancer Centre, I have experience being in the position of a project developer (in that case, Garvan) and having to address the risks faced by others (in that case, VCCRI).

7 Based on my experience, the proposed conditions of development consent are inadequate.

8 **Vibration monitoring during construction**: Proposed condition B52(c) states that vibration caused by construction must be limited to 50 micrometres per second. This limit was used by Garvan in relation to the Kinghorn Cancer Centre. This limit is meaningless unless there are adequate mechanisms to monitor vibrations and ensure that the limit is complied with. An adequate program must include:

a. Real time alert and alarm notifications. The warning alerts should be at 25 micrometres per second and the stop work alarms at 50 micrometres per second. These thresholds are the same as those recommenced in the GHD Letter (see page 6). The notifications should be distributed at ABR and on the project site, by SMS and also visually (with strobe lights) for those working machinery. These are the systems that Garvan used in relation to the Kinghorn Cancer Centre.

b. Sensors in each of: (i) the animal holding area; (ii) the mouse accommodation area; and (iii) the laboratory in which embryo microinjection occurs. These locations are the same as those recommenced in the GHD Letter (see page 6). Given their size, there would need to be two sensors in each of the animal holding area and the mouse accommodation area (one at each end).

c. Monitoring throughout the construction work.

d. Accelerometers (PCB Type 352068 and MNF Type KS943); and Sinus Harmonie 4-channel analysers integrated into computers.

9 The cost of obtaining, installing and maintaining the necessary sensors and other equipment should be borne by Plasrefine. In relation to the Kinghorn Cancer Centre, Garvan bore these costs in relation to VCCRI.

10 **Vibration limits and monitoring during operations**: As noted above, proposed condition B52(c) states that vibration caused by construction must be limited to 50 micrometres per second. It is not only necessary that this limit be observed during the construction phase of a project, but also during its operational phase. None of the proposed conditions requires that this limit be observed once the Plasrefine facility is operational. Based on my experience, it would be necessary to continue monitoring vibration levels for a period of time that is representative of ‘normal state’ after the facility is operational.

11 The costs associated with ongoing monitoring should be borne by Plasrefine.

12 **Fire risk**: Air quality is of critical importance to the health and wellbeing of mice. The air-handling equipment at ABR is not equipped to filter chemical contaminants in the air. I understand from Dr Kingham's statement that, in the event of a fire at the Plasrefine facility, the ventilation system at ABR would have to be switched off. I understand that, in those circumstances, a high level of mortality would be expected within 48 hours. I also understand that it would not be possible to relocate all of the mice at ABR in the event of a fire. The risk of loss of the mice in the event of a fire at the Plasrefine facility can only be averted by installing a suitable contamination filtration system at ABR (normally, a carbon filter is required for airborne chemical risk). A study would need to be undertaken to determine the precise filtration system necessary for ABR. In my experience, the cost of purchasing such a filtration system is at least $500,000 and the maintenance costs are $20,000 to $50,000 per year.

13 These costs should also be borne by Plasrefine.

David Keenan

Date: 2 December 2024